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DEPARTMENT OF HEALTH

CITY OF NEW YORK

DR. WILLIAM H. PARK, DIRECTOR

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In the following pages have been collected all the papers published from the Research Laboratory in the laboratory year 1908-9, as well as a number of reports and protocols which were unsuited for publication in the regular technical journals. The recipients of this volume will confer a favor on their colleagues of the Research Laboratory by sending their own publications in exchange. Such pamphlets should be addressed to the Librarian, Research Laboratory, Foot of East Sixteenth Street, New York.

THE EDITOR.

New York, September, 1910.

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THE PERCENTAGE OF CASES OF TUBERCULOSIS DUE TO THE HUMAN AND BOVINE TYPES OF BACILLI

A Study of the Tubercle Bacilli Cultivated from Four Hundred and Fifty Consecutive Cases of Human and Animal Tuberculosis.

A PRELIMINARY REPORT*

BY

Wm. H. Park, Charles Krumwiede, Jr., Elise L'Esperance, Wm. C. Thro, Bertha Van H. Anthony, Wm. H. Woglom, Frederick Montgomery.

Assisted by Louisa P. Blackburn and Angeline Courtney.

The present investigation was begun with the knowledge that investigators had already demonstrated in series of *selected* cases the fact that the bovine type of tubercle bacilli had produced a number of cases of human tuberculosis. This having been determined, it seemed to us that the most promising field for further information was to test a large series of consecutive cases, in both children and adults, *in which no selection was made.* In this way it was hoped that a fair idea of the percentage of human and bovine types of infection, in human tuberculosis, could be determined. One series of cases from the Babies Hospital we consider of special importance because in these very young infants there could be no possibility of a change of type. Another series of cases from St. Mary's Hospital, in which the type of bacilli in a number of chronic glandular cases was determined, is of great value in demonstrating that a persistence of several years in man was not sufficient to alter the characteristics of the bovine type. The results obtained by us refer only to New York City and vicinity, but in a general way they probably apply to other American cities, where the conditions are, at least in many respects, similar.

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*Four of the bacteriologists engaged in this investigation were appointed under the authority of the Commission for the Investigation of Acute Respiratory Diseases. To Drs. Hermann M. Biggs and L. Emmett Holt our thanks are especially due for their interest and assistance in carrying on the work. We are also greatly indebted to those who have made this investigation possible by their help in supplying us with the material. The full report will be in the next volume.

The report which is here submitted deals only with the most important tests, but the results obtained have been so uniform that we believe there will be little change when all the experiments have been completed.

Methods of Isolation—No direct cultures were attempted. All material was injected into guinea pigs, which were allowed to live from three to five weeks. At the end of this period the tuberculous organs were removed and thoroughly minced with knife and forceps. This minced tissue was then smeared over the surface of the culture tube. This mincing and rubbing of the material over the culture media we have found preferable to simply cutting the tissue, placing it on the surface of the media and subsequently moving to spread the growth as advised by Theobald Smith. The second opening of the tube, a fruitful source of contamination with moulds, is avoided, and the final growth is equal and greater in amount.

Methods of Cultivation—Egg media of two types have been used for isolation and further cultivation. The ease and simplicity of preparation and the uniformity of the final product make other media for isolation unnecessary.

The egg media were made as follows: Plain egg (Dorset). The whole egg was mixed with 10 per cent. by weight of water and inspissated at 70 degrees C. for two hours in a closed chamber. A few drops of sterile water were finally added to each tube to provide the necessary moisture. Glycerin egg (Lubenau) consisted of ten eggs mixed with 200 c.c. of 5 per cent. glycerin bouillon, 1.5 per cent. acid to phenolphthalein. The eggs were quite fresh. This last point we consider to be a very important detail.

Other media, as glycerin agar, glycerin bouillon and glycerin potato, were also used to determine the cultural characteristics and for further cultivation. The glycerin potato has been the most uniformly successful as a stock medium for further cultivation. The cotton stoppers in each case, with or without dipping in paraffin, were pushed down the tube, which was then closed with a tight fitting charred cork and incubated in the inclined position.

Nearly all the cultures so far isolated have fallen into two distinct groups, eugonic (luxuriant) and dysgonic (sparse). This difference is noticed on plain egg, but to a less marked degree than on glycerin egg. Here the influence of glycerin on the growth of the two types, as noticed by several investigators and carefully elaborated by Cobbett, causes a wide gap in the amount and rapidity of growth of the two types. The dysgonic type commonly fails to grow in glycerin egg from animal tissue, and if transplanted from plain egg to glycerine egg, after the first or second generation, grows but sparsely. The growth consists of thin, flat, spreading colonies 1 to 2 m.m. in diameter, or of a confluent thin, flat, slightly granular, non-pigmented layer, which is usually moist. The eugonic type, on the other hand, grows luxuriantly in most instances on glycerin egg, directly from the animal tissue, and in every case in the second or third generation. The growth is confluent, raised and crumpled or coarsely verrucose, dry, and in practically every case shows a pink pigmentation in early generations. The pink color depends on certain unknown differences in the different batches of media.

The other media used, as glycerin agar, potato and bouillon, we have found mainly of value as corroboration of the above differences for the following reason. The eugonic strains grow luxuriantly upon these media usually in the second generation and with few exceptions in the third and fourth. The dysgonic strains on the other hand usually fail to grow in the early generations, or if they do the growth is very slight.

The differences in the growth of the two types as given only apply to the first few generations, and to cultures which are three weeks old, the rapidity of growth as well as the amount being an important factor. In later generations and with longer periods of incubation the differences are not so extreme and in a few cultures the gap is closing.

The above description and division into distinct and widely separated types of growth is true of the *great majority* of cultures. There are, however, as would be expected in such closely related varieties of the same species of organism, a smaller number of cultures which tend to bridge the gap between these diverging cultural types. At the present time the following classification seems warranted, though further study may make some changes necessary.

The grades of growth here stated refer to the total amount as judged by inspection of the cultures. In each case the best growing tube of any set was considered the typical one. Nine grades were arbitrarily selected: thus Grades 1 to 3 sparse, Grades 4 to 6 moderate and Grades 7 to 9 vigorous. The reason for the selection of glycerin egg has already been spoken of. Besides albuminous media of this type we wished also to select some medium which would contain no coagulable albumins to serve as a more rigorous index of the saprophytism of the two types. After numerous trials potato was found to be the most uniform in its results. In some of the earlier cultures before this routine was established, other media, as glycerin agar and bouillon, were used in the early generations, and in these few instances we shall consider them as falling into one of the groups as though potato had been used, as they seem to be absolutely typical in other respects.

Human Type—Group 1. Glycerin egg, Grade 7 to 8 in the first three generations and pigment on glycerin egg mixtures in the early generations. Glycerin potato, Grade 6-8 in the first four generations.

Group 2. Glycerin egg, same as Group 1. Glycerin potato (a) less than Grade 6, or (b) negative or slight, in first four generations.

Group 3.* Glycerin egg. Grade 4 to 6 with pigment slight or absent in first three generations. Glycerin potato, Grade 3 to 5 in first four generations.

Group 4. Glycerin egg, as in Group 3. Glycerin potato, negative or practically so in first four generations.

Bovine Type—Group 1. Glycerin egg, Grade 0 to 3 for first three generations; usually negative or slight directly from the animal tissue and growth remaining moderate for at least five† generations, viz: not increasing above Grade 6. Glycerin potato, slight or negative in the first four generations.

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* No culture of this group is included in the series here reported.

† The limit had been originally set at ten generations, but at the time of correcting proof it was found preferable to change this to five, to throw Group 3 into greater relief. These five generations refer to transfers on glycerine egg, preceding glycerine free media not counted.

Group 2. Glycerin egg, Grade 4 in first three generations. That is, the growth is better than Group 1, but increases slowly in amount in the first five generations. Glycerin potato, slight or very moderate growth in the first four generations.

Group 3. At first, as in Group 1 on both glycerin egg and potato, but rapidly increases in amount of growth in the first five generations, reaching Grade 7, and showing some pigmentation on reaching the higher grades.

When one studies this grouping of the two types there is apparent a gradual gradation from one type to the other. Of the human types no one could mistake Groups 1 and 2 for bovine cultures, nor could Group 1 of the bovine type be mistaken for human cultures, if one had handled even a few cultures on these media. As to Group 3 of the human type, the difference is still sufficiently marked to make a decision as to type on inspection of the tubes of the first few generations. When Group 4 is reached, differentiation is very difficult on these two media. Other media may add some information in these cases, as for instance the ability to grow from glycerin egg on to glycerin bouillon added to the tube, a phenomenon we have not seen in any early bovine culture. Group 3 of the bovine type has been differentiated without exception by the characteristics of the early generations, though when the first culture of this type was encountered we considered the possibility of error. Further observation, however, has shown that the early generations, as we thought, are the diagnostic ones. After a few more generations the distinctions in human Groups 3 and 4 and bovine Group 3 are lost and the last tends to outstrip the other two in vigor of growth. These three groups with Group 2 of the bovine type then tend to bridge the gap and form a complete series of types.

This division into two types according to the cultural characteristics has been uniform with the results of animal inoculations, and in practically every case the rabbit virulence has been correctly predicted from the cultural characteristics.

The following is the method we have employed in testing the virulence on rabbits:

The bacilli are removed from the surface of a subculture which is less than one month old and the excess of moisture removed by gentle pressure between sterile filter papers before the mass is weighed. The culture medium used for these subcultures, with a few exceptions, has been glycerin egg, and there have been added to the tubes after coagulation of the medium a few drops of glycerin bouillon. The growth immediately above the fluid can be readily emulsified. After weighing, the bacterial mass is emulsified in physiological salt solution, which is added in such amount that 1 c.c. of emulsion represents 1 mg. of organisms. The dysgonic viruses in many instances afforded too little growth for weighing. An emulsion was therefore made and compared with a known emulsion from a eugonic virus, the comparison consisting in the equal ease with which print could be read through each emulsion. A slight amount of saline solution was then added, in order that the error, if any, might fall upon the small side. The intravenous route was decided upon as the most suitable for our purpose, and it has been adhered to throughout the experiment. The dose used has been 1 mg. or .01 mg. With the latter dose the stock suspension was diluted with saline, the amount of the fluid injected being always 1 c.c.

It soon became evident that we had to deal with two varieties of organisms, one of which far exceeds its fellow in virulence. An animal inoculated intravenously with 1 mg. or with .01 mg. of bacilli of the bovine type soon becomes emaciated, the fur loses its lustre, respiration becomes labored and death occurs with the larger dose in from 17 to 38 days, with an average period of 21 days, and with the smaller dose in from 25 to 78 days, with an average of 44 days. Autopsy reveals extreme emaciation and except in rare instances* a generalized tuberculosis involving almost every structure in the body. The lesions in the lymph nodes, and of these particularly the inguinal and the axillary nodes are almost pathognomic. The nodes are enlarged, congested and studded with minute caseous areas. The spleen is three or four times its normal size and riddled with tubercles, and the kidneys are sure to contain on their surfaces an average of from six to ten tubercles each. The liver very commonly contains macroscopic tubercles.

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* There were four of these rabbits in which the lesions were localized chiefly in the lungs. The same cultures used in the smaller dose produced generalized tuberculosis.

The lymph nodes of the abdomen are usually enlarged, as are those of the thorax, and commonly show small tubercles which may be caseous. The lungs are always largely consolidated, and are literally a mass of tubercles. Tubercles in the heart muscle are not infrequent. The duration of the illness is shortened by increase of the dose and is also influenced by the individual resistance of the animals. Old rabbits do not seem to be more resistant than young ones.

Separated from such viruses by a wide interval of virulence, are the eugonic viruses. These never cause generalized progressive lesions in doses of .01 mg. or even 1 mg. and a rabbit so inoculated will usually live until carried off by an intercurrent malady. The appearance at autopsy is in striking contrast to the findings described in the preceding paragraph. The animal will be found well provided with subcutaneous and abdominal fat, and the lymph nodes, with few exceptions, are normal in size and appearance. The appearance of the lungs varies with the length of time that has elapsed since inoculation. Six weeks after injection they are pretty extensively involved, for at this point, of course, many of the inoculated organisms are arrested. But that the lesions under discussion are regressive is shown by the fact that many animals have gained weight, as well as by our observation that in rabbits, autopsied after an interval of three or four months, the lungs are pink, well collapsed and with tubercles only sparingly distributed throughout them. Almost always there are a few tubercles in the kidneys. The spleen is of normal size, and it is very rare that a tubercle may be found on its surface. Tubercles in the heart muscle are quite uniformly absent. Occasionally after a long interval there occurs chronic tuberculous-like lesions, such as orchitis, osteomyelitis, adenitis, mastitis or cold abscess.*

As in the cultural classification, so in the virulence as tested on rabbits, the great majority of cultures are included in two widely divergent types. There are, to be sure, some showing intermediate grades of virulence. The viruses which show this variation in virulence have not yet been sufficiently studied to warrant any subdivision of groups as in the cultural characteristics. We are, therefore, not in a position to say how far there will be a tendency to fill the gap between the two extremes of virulence. We can say, however, that these variations have not given us any reason to doubt the reliability of the virulence test in rabbits as a means of differentiation when the test is properly controlled by repetition where any variation occurs in one or more rabbits of a series.

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* Although these lesions were considered tuberculous at first, later observations have thrown doubt on this interpretation.

Wherever any intermediate characteristics of cultures or rabbit virulence have occurred, the combined evidence of both left no doubt as to the type of organism.

In a series of selected cultures we have tested the virulence on young calves. Fifty mgs. of culture emulsified in 5 c.c. of normal saline were injected subcutaneously in the neck. Here again the cultures have fallen into two distinct groups. The human type caused only a local lesion, or at most an extension into the neighboring lymph nodes without producing any effect on the health of the calf. The bovine type, on the other hand, produced an acutely fatal generalized tuberculosis. Varying from these two extremes was the result in one calf receiving a bovine virus, where a chronic and apparently regressive generalized tuberculosis was caused. At the end of this article are given the results in each calf inoculated and the viruses used.

The above description has rather accentuated the variations from the widely separated characteristics of the two types. We have done this to show plainly the fallacy of working with a few cultures and then questioning the existence of two distinct types of tubercle bacilli. This would probably be the result if an investigator should happen upon several variants in a small series of cultures. On the other hand if, as might readily happen, all cultures were absolutely typical of the two extremes his conclusions would be diametrically opposite. In neither case would his conclusions be true. Our own ideas have been modified from time to time, the earlier work pointing to wide divergence of types only, the latter work showing variations from these results. This is really what one would expect from analogy with other bacteria, viz., that in two closely related types of organism there is not a wide gap, but rather two norms around which the two types vary, these variants in no way causing any doubt as to the existence of the two types.

The following tables give the cases investigated with the results of cultural characteristics and rabbit virulence, according to the foregoing descriptions. Wherever there are variations from the extreme types, a detailed description of this virus will be given in the final report of our work, this being impossible at the present time.

The rather arbitrary division of cases under sixteen years from the older cases was adopted, as can be seen from the summary of results, to throw the bovine types mainly into one class and to show clearly the age incidence of these cases.

PULMONARY TUBERCULOSIS—ADULTS

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.*	Diagnosis.
5	Sputum	H. Type 1 †	Non-virulent	A.K.	17 yrs.	F.	1½ yrs.	Pulmonary Tuberculosis
9	"	"	"	J.W.	22	M.	7 mos.	"
10	"	"	"	M.S.	adult	F.	?	"
11	"	"	"	M.S.	16 yrs.	F.	?	"
13	"	"	"	R.R.	adult	?	?	"
22	"	"	"	M.V.	24 yrs.	F.	?	"
26	"	"	"	W.E.	adult	?	?	"
27	"	"	"	A.M.	38 yrs.	?	?	"
32	"	"	"	G.K.	adult	F.	?	"
34	"	"	"	H.W.	adult	M.	2 yrs.	"
35	"	"	"	A.M.	30 yrs.	F.	3 "	"
36	"	"	"	A.S.	adult	?	7–8 mos.	"

(5) Lesions. "Bronchitis" and subacute pleurisy.

(9) Lesions. Infiltration of right, lower and upper lobes, beginning consolidation. Prognosis unfavorable.

(10) No details obtainable.

(11) Case reported in history as chronic endocarditis. No sputum examination recorded.

(13) No details obtainable.

(22) Incipient stage. No further information obtainable.

(26) "Large lesions in left lung" is the only information that can be elicited.

(27) No details obtainable.

(32) No details obtainable.

(34) Lesions. Involvement of both apices. Prognosis good. Looks perfectly well.

(35) Lesions. Old lesion in right apex, infiltration of left apex. Prognosis favorable.

(36) Lesions. Both lungs and pleura, "severe."

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* Duration of disease to time specimen was received.

† H. type 1 means Human type, group 1, etc. See explanation, page 10.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
37 124	Sputum "	H. Type I "	Non-virulent "	J.B.	19 yrs.	M.	2½ yrs.	Pulmonary Tuberculosis
38	"	"	"	B.P.	adult	?	?	"
39	"	"	"	J.H.	24 yrs.	M.	1½ yrs.	"
40	"	"	"	M.S.	adult	?	?	"
41	"	"	"	A.H.	"	F.	?	"
42	"	"	"	J.W.	"	M.	3 mos.	"
43	"	"	"	D.K.	33 yrs.	M.	2 "	"
44	"	"	"	E.K.	39 "	M.	5 yrs.	"
46	"	"	"	L.T.	40 "	M.	1 yr.	"
47	"	"	"	M.F.	adult	M.	"several mos."	"
48	"	"	"	W.B.	29 yrs.	M.	4 yrs.	"
49	"	"	"	L.L.	35 "	M.	6 mos.	"

(37) Lesions. Infiltration both apices, both upper lobes involved. Prognosis poor.

(38) No details obtainable.

(39) Lesions. Infiltration right apex and left lung. Cervical adenitis. Lymph nodes removed two years ago.

(40) No details obtainable.

(41) No details obtainable.

(42) Lesions. Both apices and upper part of lobes.

(43) Lesions. Infiltration consolidation and cavity.

(44) Lesions. Both apices, cavity in left apex? Cervical nodes enlarged. Prognosis bad.

(46) Lesions. Both apices and part of right upper lobe consolidated. Prognosis fair.

(47) Lesions. Apices, slight.

(48) Lesions. Second stage. Apices chiefly affected, no cavities. Prognosis poor.

(49) Lesions. Consolidation of right upper lobe. On the left side, infiltration from apex down to the third space.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
51	Sputum	H.Type 1	Non-virulent	J.G.	30 yrs.	M.	1 yr.	Pulmonary Tuberculosis
52	"	"	"	L.G.	18 "	M.	2 mos.	"
53	"	"	"	W.Q.	28 "	M.	3 "	"
54	"	"	"	M.K.	42 "	M.	3 "	"
55	"	"	"	J.S.	28 "	M.	6 "	"
56	"	"	"	L.F.	28 "	F.	?	"
57	"	"	"	J.M.	44 "	M.	3 mos.	"
59	"	"	"	?	adult	?	?	"
60	"	"	"	J.D.	45 yrs.	M.	1 yr.	"
64	Adenoids and Tonsils	"	"	T.W.	20 "	M.	8 mos.	"
125	Sputum	"	"					
75	"	"	"	S.T.	19 "	M.	6 wks.	"
76	"	H.Type2b	"	A.D.	21 "	M.	6 mos.	"
77	"	H.Type 1	"	M.S.	18 "	M.	4 "	"

(51) Lesions. Right side, apex to third rib involved.

(52) Lesions. Diffuse infiltration right lung, left apex and upper lobe. Prognosis poor.

(53) Lesions. Diffuse infiltration left lung and right apex and upper lobe.

(54) Lesions. Consolidation left apex and upper lobe. Beginning infiltration of right apex.

(55) Lesions. Disseminated infiltration left lung, and right apex and upper lobe. Cervical nodes enlarged.

(56) Lesions. Infiltration, right lung.

(57) Lesions. Infiltration of right apex. Prognosis good.

(59) Lesions. No details obtainable.

(60) Lesions. Greater part of right lung consolidated. Infiltration of left upper lobe. Axillary nodes enlarged.

(64) Lesions. Disseminated infiltration of left lung. Prognosis good.

(75) Lesions. Infiltration of right apex and upper lobe. Few small areas of infiltration in left lower lobe.

(76) Lesions. Infiltration of both upper, and upper portions of both lower lobes. General pleural involvement.

(77) Lesions. "Flatness over lower part of right chest, probably fluid." Prognosis bad.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
78	Sputum	H.Type 1	Non-virulent	J.R.	46 yrs.	M.	?	Pulmonary Tuberculosis
79	"	"	"	J.M.	19 "	F.	4 mos.	"
80	"	"	"	D.F.	37 "	M.	10 "	"
81	"	"	"	C.H.	36 "	M.	6 "	"
82	"	"	"	E.B.	29 "	M.	8 "	"
83	"	"	"	L.B.	42 "	M.	3 yrs.	"
84	"	"	"	J.M.	49 "	M.	6 mos.	"
85	"	"	"	C.B.	34 "	M.	2 yrs.	"
86	"	"	"	M.L.	30 "	M.	6 mos.	"
87	"	"	"	M.Y.	28 "	M.	3 "	"
88	"	"	"	H. S.	29 "	M.	3 "	"
90	"	"	"	A.F.	58 "	M.	7 "	"
92	"	"	"	A.N.	adult	F.	6 "	"
101	"	"	"	H.H.	20 yrs.	M.	9 yrs.	"

(78) Lesions. Extensive infiltration of both lungs. Axillary and cervical nodes enlarged.

(79) Lesions. Infiltration of right apex and part of upper lobe "cavity."

(80) Lesions. Infiltration of right apex. Axillary and cervical nodes enlarged. Prognosis fair.

(81) Lesions. Consolidation both lungs. Prognosis bad.

(82) Lesions. Infiltration both apices and upper lobes, small portion of lower lobes. Axillary and cervical nodes enlarged. Prognosis doubtful.

(83) Lesions. Both lungs involved. Prognosis fair.

(84) Lesions. Infiltration of left apex.

(85) Lesions. Infiltration of both apices.

(86) Lesions. Consolidation, right apex and upper lobe. Left apex, infiltrated areas.

(87) Lesions. Both upper lobes involved.

(88) Lesions. Consolidation left apex and part of upper lobe.

(90) Lesions. Consolidation both apices and upper lobes. Prognosis bad.

(92) Lesions. Infiltration of apices.

(101) Lesions. Complete infiltration, much consolidation.

PULMONARY TUBERCULCSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
102	Sputum	H. Type 1	Non-virulent	M.G.	29 yrs.	M.	1 yr.	Pulmonary Tuberculosis
103	"	"	"	H.C.	25 "	F.	5 mos.	"
104	"	"	"	P.P.	22 "	M.	2 yrs.	"
105	"	"	"	M.M.	23 "	M.	1 yr.	"
106	"	"	"	L.C.	24 "	F.	6 mos.	"
108	"	"	"	P.Z.	22 "	M.	1 mo.	"
110	"	"	"	D.S.	34 "	M.	1 yr.	"
112	"	"	"	S.J.	23 "	M.	8 mos.	"
113	"	"	"	M.E.	27 "	F.	2 yrs.	"
114	"	"	"	P.R.	45 "	M.	6 mos.	"
117	"	"	"	M.R.	17 "	F.	5 "	"
118	"	"	"	A.W.	35 "	F.	1 yr.	"
119	"	"	"	R.R.	33 "	M.	10 yrs. ?	"
120	"	"	"	J.S.	39 "	M.	6 mos.	"
121	"	"	"	C.O.	37 "	M.	2 "	"
124	See 37							

(102) Lesions. Infiltration upper lobes, and upper part of right lower. Axillary and cervical nodes enlarged.

(103) Lesions. Consolidation right apex.

(104) Lesions. Infiltration both upper and upper part of lower lobes. Axillary and cervical nodes enlarged.

(105) Lesions. Infiltration left apex and upper lobe beginning involvement of right apex.

(106) Lesions. Consolidation of right lung. Prognosis bad.

(108) Lesions. Infiltration of left lower and part of upper lobes. Prognosis fair.

(110) Lesions. Extensive infiltration, both lungs. Axillary and cervical nodes enlarged.

(112) Lesions. Diffuse infiltration of left lung. Prognosis fair.

(113) Lesions. Infiltration left upper lobe. Prognosis unfavorable.

(114) Lesions. Consolidation both lungs with cavities in right upper and left lower lobes. Prognosis bad.

(117) Lesions. Infiltration, entire lungs. Left apex most marked. Few cervical nodes enlarged.

(118) Lesions. No details obtainable. Prognosis unfavorable.

(119) Lesions. Involvement of right apex, upper and middle lobe, also left upper lobe.

(120) Lesions. Consolidation both apices and upper lobes, areas of infiltration.

(121) Lesions. Consolidation, right lung and left apex and upper lobe.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis
125	See 64							
126	Sputum	H.Type 1	Non-virulent	J.W.	22 yrs.	M.	3 mos.	Pulmonary Tuberculosis
128	"	"	"	I.W.	42 "	M.	6 "	"
131	"	"	"	V.G.	16 "	F.	2 "	"
133	"	"	"	J.D.	18 "	M.	4 "	"
134	"	"	"	R.H.	27 "	M.	4 "	"
135	"	"	"	D.B.	44 "	F.	2 yrs.	"
136	"	"	"	F.O.	18 "	F.	1 yr.	"
219	"	"	"	P.McC.	23 "	M.	2 yrs.	"
220	"	"	"	J.C.	27 "	M.	14 "	"
221	"	"	"	A.H.	38 "	M.	20 "	"
222	"	"	"	R.L.	30 "	M.	4 "	"
223	"	"	"	W.M.	47 "	M.	3 "	"

(126) Lesions. Extensive infiltration both lobes. Cavity in left lower lobe. Axillary and cervical nodes enlarged. Prognosis bad.

(128) Lesions. Both lungs involved. Prognosis fair.

(131) Lesions. Infiltration and consolidation both apices. Infiltration both upper lobes.

(133) Lesions. Diffuse infiltration both lungs, marked enlargement of cervical nodes.

(134) Lesions. Consolidation of right apex and upper lobe. Laryngitis.

(135) Lesions. Consolidation of right apex, infiltration left upper lobe. Prognosis fair.

(136) Lesions. Infiltration of right apex and upper lobe. Cavity in upper lobe. Prognosis fair.

(219) Lesions. Consolidation of right apex and upper lobe, slight involvement of middle lobe, and of left apex posteriorly. Progress acute.

(220) Lesions. Partial consolidation of right apex and upper lobe with slight involvement of left upper lobe. Progress acute.

(221) Lesions. Scattered areas of consolidation in both lungs. Progress acute.

(222) Lesions. Slight involvement of both apices. Generalized bronchitis.

(223) Lesion. Consolidation of right apex and in upper left lobe. Progress subacute with exacerbation.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
224	Sputum	H.Type 1	Non-virulent	J.B.	54 yrs.	M.	2½ mos.	Pulmonary Tuberculosis
227	"	"	"	F.W.	20 "	M.	4 "	"
228	"	"	"	C.A.	18 "	M.	2 "	"
229	"	"	"	J.L.	32 "	M.	13 "	"
230	"	"	"	S.K.	18 "	M.	2 "	"
231	"	"	"	J.A.	21 "	M.	4 "	"
232	"	"	"	D.M.	36 "	M.	9 "	"
233	"	"	"	F.C.	22 "	M.	1 yr.	"
234	"	"	"	M.D.	24 "	M.	8 mos.	"
235	"	"	"	G.B.	36 "	M.	6 "	"
237	"	"	"	W.R.	51 "	M.	9 "	"
240	"	"	"	J.K.	29 "	M.	8 "	"
241	"	"	"	J.H.	38 "	M.	9 "	"
242	"	"	"	J.B.	49 "	M.	1 yr.	"

(224) Lesions. Fibroid right lung. Scattered areas in left lung. Progress chronic.

(227) Lesions. Consolidation of both apices. Progress acute.

(228) Lesions. Consolidation of left apex and upper lobe. Progress acute.

(229) Lesions. Consolidation of right apex and upper lobe and of left lower lobe. Progress chronic.

(230) Lesions. Scattered areas of dullness throughout both lungs. Progress acute.

(231) Lesions. Scattered areas throughout both lungs. Cavity in left lower lobe. Progress acute.

(232) Lesions. Both apices consolidated, involvement of left lobe. Progress chronic.

(233) Lesions. Involvement of both apices and left lower lobe. Progress acute.

(234) Lesions. Consolidation of right apex and upper lobe.

(235) Lesions. General involvement. Progress acute.

(237) Lesion. Fibroid left upper lobe. Infiltration of right upper lobe. Progress chronic.

(240) Lesions. Right apex and upper lobe consolidated. Progress subacute.

(241) Lesions. Involvement of both apices and upper lobes. Progress acute.

(242) Lesions. Involvement of both upper lobes. Progress subacute.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
243	Sputum	H.Type 1	Non-virulent	H.H.	22 yrs.	M.	3 yrs.	Pulmonary Tuberculosis
244	"	"	"	M.K.	29 "	M.	1 mo.	"
245	"	"	"	J.K.	23 "	M.	3 yrs.	"
246	"	"	"	M.S.	50 "	M.	4 wks.	"
248	"	"	"	T.H.	29 "	M.	18 mos.	"
249	"	"	"	D.A.	24 "	M.	6 "	"
250	"	"	"	A.B.	28 "	M.	2½ yrs.	"
251	"	"	"	E.M.	17 "	M.	2 "	"
252	"	"	"	L.E.	26 "	M.	4 mos.	"
253	"	"	"	J.C.	39 "	M.	1 yr.	"
254	"	"	"	J.L.	18 "	M.	16 mos.	"
255	"	"	"	A.R.	50 "	M.	13 "	"
256	"	"	"	E.J.	28 "	M.	2½ yrs.	"
280	"	"	"	M.S.	33 "	F.	5 "	"

(243) Lesions. Involvement of right apex and upper lobe. Scattered areas, lower lobes. Progress acute.

(244) Lesions. Left apex and upper lobe, right upper lobe posteriorly consolidated. Progress acute.

(245) Lesions. Right apex, middle lobe and left base involved. Progress acute.

(246) Lesions. Right upper lobe and lower lobe posteriorly consolidated. Progress acute.

(248) Lesions. Consolidation of right apex. Progress acute.

(249) Lesions. Consolidation of right apex, and upper part of middle lobe. Progress acute.

(250) Lesions. Right apex and upper lobe and left apex involved. Progress chronic.

(251) Lesions. Left apex and middle lobe involved.

(252) Lesions. Consolidation of both apices and right upper lobe. Progress acute.

(253) Lesions. General dullness. Cervical nodes on right side enlarged. Progress chronic.

(254) Lesions. Consolidation left apex and upper lobe. Progress chronic.

(255) Lesions. Consolidation right apex and upper lobe.

(256) Lesions. Extreme involvement of right lungs. Dullness of left base. Progress chronic.

(280) Lesions. Infiltration both apices and upper lobes. Prognosis fair.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
285	Sputum	H.Type 1	Non-virulent “	S.H.	19 yrs.	M.	4 mos.	Pulmonary Tuberculosis
286	“	“	“	A.R.	34 “	M.	1 mo.	“
287	“	“	“	J.M.	31 “	M.	9 mos.	“
288	“	“	“	W.M.	38 “	M.	8 yrs.	“
289	“	“	“	F.K.	19 “	F.	5 mos.	“
290	“	“	“	G.W.	39 “	M.	2 yrs.	“
294	“	“	“	O.F.	22 “	M.	6 mos.	“
295	“	“	“	H.N.	36 “	F.	1 mo.	
367	“	“	Not tested					
296	“	“	Non-virulent	H.G.	30 “	M.	10 mos.	“
298	“	“	“	W.	50 “	?	6 “	“
300	“	“	“	B.L.	36 “	F.	8 “	“
302	“	“	“	B.M.	53 “	M.	3 “	“
303	“	“	“	E.B.	37 “	M.	3 yrs.	“

(285) Lesions. Left apex and upper lobe and upper part of lower lobe involved. Prognosis fair.

(286) Lesions. Consolidation of right apex and part of upper lobe. Infiltration of left apex. Prognosis fair.

(287) Lesions. Infiltration right upper lobe. Prognosis poor.

(288) Lesions. Consolidation right apex and upper lobe, and left apex. Prognosis good.

(289) Lesions. Disseminated infiltration of left lung. Beginning infiltration of right apex. Prognosis fair.

(290) Lesions. Infiltration of right apex. Area of consolidation in upper posterior part of left lower lobe. Prognosis fair.

(294) Lesions. Right apex and upper lobe involved.

(295) Lesions. Infiltration left apex and upper lobe.

(296) Lesions. Infiltration both apices.

(298) Lesions. Consolidation of right apex. Infiltration of right upper lobe and left lower lobe.

(300) Lesions. Right apex and upper lobe involved.

(302) Lesions. Right apex involved.

(303) Lesions. Whole right lung and right apex involved.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
304	Sputum	H.Type 1	Non-virulent "	J.C.	41 yrs.	M.	1 yr.	Pulmonary Tuberculosis
305	"	"	"	F.R.	18 "	M.	7 wks.	"
307	"	"	"	E.G.	38 "	M.	1 yr.	"
337	"	"	"	R.R.	16 "	F.	4 mos.	"
338	"	"	"	T.Van N	19 "	F.	6 "	"
339	"	"	"	B.D.	29 "	F.	1 yr.	"
340	"	"	"	M.M.	20 "	F.	1 mo.	"
341	"	"	"	R.I.	32 "	M.	2 yrs.	"
344	"	"	"	H.E.	18 "	M.	2½ "	"
345	"	"	"	P.H.	42 "	M.	13 mos.	"
346	"	"	"	G.G.	35 "	M.	1½ yrs.	"
347	"	"	"	L.C.	24 "	M.	1½ "	"
348	"	"	"	D.L.	46 "	M.	1 yr.	"
349	"	"	"	G.E.	53 "	M.	7 mos.	"
350	"	"	"	E.B.	24 "	M.	4 "	"
351	"	"	"	J.M.	41 "	M.	2¼ yrs.	"
352	"	"	"	B.R.	28 "	M.	2 "	"

(304) Lesions. Extensive infiltration and softening of left lung.

(305) Lesions. Right apex and upper lobe involved.

(307) Lesions. Extensive infiltration of right lung; slight infiltration of left apex. Prognosis poor.

(337) Lesions. At first reported infiltration right apex; prognosis good. Later reported "no case." Three sputum examinations negative.

(338) Lesions. Infiltration of both apices. Prognosis good.

(339) Lesions. Infiltration of right apex and upper lobe and of left apex. Prognosis fair.

(340) Lesions. Infiltration left lower lobe and slight infiltration of left apex. Prognosis good.

(341) Lesions. Second stage. Progressive.

(344) Lesions. Second stage. Bone necrosis of right elbow. Improving.

(345) Lesions. Second stage. Improving.

(346) Lesions. Third stage. Progressive.

(347) Lesions. Third stage. Improving.

(348) Lesions. Second stage. Progressive.

(349) Lesions. Third stage. Progressive.

(350) Lesions. Third stage. Progressive.

(351) Lesions. Second stage. Progressive.

(352) Lesions. Third stage. Progressive.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
353	Sputum	H.Type 1	Non-virulent "	F.L.	34 yrs.	M.	5 mos.	Pulmonary Tuberculosis
354	"	"	"	H.S.	26 "	M.	6 "	"
355	"	"	"	J.D.	46 "	M.	3½ yrs.	"
356	"	"	"	J.K.	31 "	M.	5½ "	"
357	"	"	"	H.H.	30 "	M.	1 yr.	"
358	"	"	"	A.Z.	30 "	M.	6 mos.	"
359	"	"	"	F.S.	18 "	M.	8 "	"
360	"	"	"	J.L.	43 "	M.	7 yrs.	"
361	"	"	"	C.C.	30 "	M.	7 mos.	
362	"	"	"	C.C.	21 "	M.	6 "	"
364	"	"	"	P.S.	42 "	M.	1 yr.	"
366	"	"	"	W.J.	30 "	M.	5½ yrs.	"
367	See 295	"	"					
368	Sputum	"	"	B.T.	48 "	M.	8 "	"
370	"	"	"	F.Z.	21 "	M.	6 mos.	"
371	"	"	"	J.M.	31 "	M.	1 yr.	"
373	"	"	"	E.G.	34 "	F.	?	"

(353) Lesions. Third stage. Progressive.
(354) Lesions. Second stage. Improving.
(355) Lesions. Third stage. Progressive.
(356) Lesions. Third stage. Improving.
(357) Lesions. Third stage. Progressive.
(358) Lesions. First stage. Improving.
(359) Lesions. Second stage. Improving.
(360) Lesions. Second stage. Improving.
(361) Lesions. Second stage. Progressive.
(362) Lesions. Second stage. Improving.
(364) Lesions. Second stage. Quiescent.
(366) Lesions. Third stage. Progressive.
(368) Lesions. Third stage. Progressive.
(370) Lesions. Third stage. Progressive.
(371) Lesions. Second stage. Improving.
(373) Lesions. Partial consolidation of both upper lobes, and infiltration of right lower. Prognosis poor.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
374	Sputum	H.Type 1	Non-virulent	P.R.	45 yrs.	M.	2 mos.	Pulmonary Tuberculosis
378	"	"	"	K.S.	39 "	M.	7 "	"
379	"	"	"	J.McG.	26 "	M.	4 "	"
380	"	"	"	F.S.	20 "	M.	11 "	"
384	"	"	"	E.M.	21 "	M.	1 mo.	"
385	"	"	"	E.H.	43 "	M.	3 mos.	"
386	"	"	"	L.W.	30 "	M.	1½ yrs.	"
387	"	"	"	G.C.	18 "	M.	5 mos.	"
390	"	"	"	G.A.	39 "	M.	8 "	"
391	"	"	"	E.H.	51 "	M.	8 wks.	"
398	"	"	"	R.H.	22 "	M.	6 mos.	"
399	"	"	"	B.McK.	73 "	M.	2¾ yrs.	"
400	"	"	"	A.P.	24 "	M.	2 yrs.	"
401	"	"	"	J.McH.	34 "	M.	1¼ yrs.	"
402	"	"	"	M.H.	42 "	M.	1⅔ "	"
403	"	"	"	M.D.	21 "	M.	7 mos.	"
404	"	"	"	J.C.	29 "	M.	1¼ yrs.	"
405	"	"	"	G.S.	37 "	M.	1 yr.	"
407	"	H.Type 2a	"	H.S.	37 "	M.	2⅔ yrs.	"

(374) Lesions. Infiltration left apex and upper lobe. Prognosis fair.
(378) Lesions. Infiltration of both apices. Prognosis fair.
(379) Lesions. Infiltration of right apex, consolidation of left apex. Prognosis poor.
(380) Lesions. Infiltration of right lung and left apex. Prognosis doubtful.
(384) Lesions. Infiltration of right apex. Prognosis fair.
(385) Lesions. Infiltration of left apex and left lower lobe. Prognosis good.
(386) Lesions. Infiltration both apices and upper lobes. Prognosis poor.
(387) Lesions. Infiltration left upper lobe, and slight infiltration of right upper and left lower lobes. Prognosis fair.
(390) Lesions. Infiltration of both apices. Prognosis good.
(391) Lesions. Consolidation of left upper lobe.
(398) Lesions. First stage. Improving.
(399) Lesions. Third stage. Progressive.
(400) Lesions. Second stage. Progressive.
(401) Lesions. Second stage. Improving.
(402) Lesions. Third stage. Progressive.
(403) Lesions. Second stage. Progressive.
(404) Lesions. Second stage. Improving.
(405) Lesions. Second stage. Improving.
(407) Lesions. Second stage. Progressive.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
410	Sputum	H.Type 1	Non-virulent	P.C.	27 yrs.	F.	7 mos.	Pulmonary Tuberculosis
412	“	“	“	W.R.	43 “	F.	10 “	“
413	“	“	“	V.E.	35 “	F.	6 “	“
414	“	“	“	W.H.	40 “	F.	12 yrs.	“
415	“	“	“	H.J.S.	49 “	F.	1⅔ “	“
417	“	“	“	T.L.	47 “	F.	1 yr.	“
418	“	“	“	J.K.	21 “	M.	3 mos.	“
419	“	“	“	N.H.	59 “	M.	3 yrs.	“
420	“	“	“	P.R.	41 “	M.	2 “	“
421	“	H.Type 2b	“	M.S.	44 “	M.	9 “	“
422	“	H.Type 1	“	M.A.	20 “	F.	3 “	“
423	“	“	“	R.L.	21 “	M.	6 mos.	“
425	“	“	“	W.H	21 “	M.	1 yr.	“
427	“	“	“	F.K.	18 “	M.	4 mos.	“
431	“	“	“	W.B.	45 “	M.	4 “	“
432	“	“	“	E.E.	22 “	M.	1 yr.	“

(410) Lesions. Second stage. Progressive.
(412) Lesions. Second stage. Progressive.
(413) Lesions. Second stage. Progressive.
(414) Lesions. Third stage. Progressive.
(415) Lesions. Third stage. Progressive.
(417) Lesions. Second stage. Improving.
(418) Lesions. Third stage. Progressive.
(419) Lesions. Third stage. Progressive.
(420) Lesions. Second stage. Progressive.
(421) Lesions. Third stage. Progressive.
(422) Lesions. Third stage. Progressive.
(423) Lesions. Second stage. Improving.
(425) Lesions. Third stage. Progressive.
(427) Lesions. Left base involved. Prognosis fair.
(431) Lesions. Infiltration of both apices. Prognosis good.
(432) Lesions. Consolidation of right upper and middle lobes. Prognosis poor.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
436	Sputum	H. Type 1	Non-virulent	J.McD.	29 yrs.	M.	10 wks.	Pulmonary Tuberculosis
437	"	H. Type 2a	"	S.A.	38 "	M.	7 mos.	"
440	"	H. Type 1	"	W.S.	39 "	M.	11 "	"
444	"	"	"	A.F.	38 "	F.	7 "	"
445	"	"	"	M.M.	37 "	F.	4 "	"
447	"	"	"	M.P.	24 "	?	?	"
448	"	"	"	M.McK.	35 "	F.	3 yrs.	"
449	"	"	"	M.M.	50 "	F.	1½ "	"
588	"	"	Not tested					
593	"	"	"					
450	"	"	Non-virulent	A.C.	44 "	F.	1½ "	"
451	"	"	"	C.B.	34 "	F.	3 mos.	"
452	"	"	"	A.Y.	22 "	F.	1 yr.	"
453	"	"	"	M.P.	42 "	F.	1½ yrs.	"

(436) Lesions. Infiltration of both apices. Prognosis fair.

(437) Lesions. Diffuse consolidation of right lung. Prognosis poor.

(440) Lesions. Infiltration of right upper lobe, left apex and left lower lobe. Prognosis fair.

(444) Lesions. Right lung and left apex involved. Progress acute.

(445) Lesions. Consolidation of right apex. Progress chronic.

(447) Lesions. Slight involvement of right lung. Condition good.

(448) Lesions. Scattered lesions in both lungs. Condition poor.

(449) Lesions. Extensive involvement of both lungs. Progress slow. Condition fair.

(450) Lesions. Involvement of both lungs. Condition fair.

(451) Lesions. Involvement of left lung. Progress slow. Condition good.

(452) Lesions. Involvement of both lungs. Course slow. Condition fair.

(453) Lesions. Involvement of left apex and right lung. Course slow. Condition fair.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
457	Sputum	H.Type 1	Non-virulent	R.M.	32 yrs.	F.	1½ yrs.	Pulmonary Tuberculosis
459	"	"	"	M.P.	60 "	F.	1 yr.	"
460	"	"	"	M.L.	46 "	F.	1½ yrs.	"
461	"	"	"	M.W.	34 "	F.	2¼ "	"
462	"	"	"	G.M.	25 "	F.	1 yr.	"
464	"	"	"	L.M.	36 "	F.	4 mos.	"
465	"	"	"	E.M.	33 "	F.	11 "	"
466	"	"	"	H.J.	45 "	F.	1½ yrs.	"
468	"	"	"	A.M.	59 "	F.	1¼ "	"
469	"	"	"	L.S.	47 "	F.	10 mos.	"
470	"	"	"	M.McN.	36 "	F.	6 "	"
472	"	"	"	A.L.	32 "	F.	3 yrs.	"
473	"	"	"	F.L.	57 "	F.	3 "	"
475	"	"	"	L.D.	adult	F.	3 "	"
476	"	"	"	B.G.	28 yrs.	F.	2 "	"

(457) Lesions. Involvement of both apices. Course slow. Condition fair.

(459) Lesions. Involvement of right upper lobe. Course slow. Condition good.

(460) Lesions. Scattered lesions in both lungs. Condition good. Course slow.

(461) Lesions. Involvement of left lung anteriorly and right base posteriorly. Course slow. Condition fair.

(462) Lesions. Involvement of both lungs. Course slow. Condition fair.

(464) Lesions. Involvement of right lung and left apex. Course slow. Condition good.

(465) Lesions. Involvement of left apex and right lung anteriorly. Course slow. Condition good.

(466) Lesions. Extensive lesions of left lung. Course slow. Condition poor.

(468) Lesions. Scattered lesions in right lung. Consolidation of left lung. Course rapid. Condition, dead.

(469) Lesions. Right upper lobe consolidated. Course slow. Condition good.

(470) Lesions. Left apex involved. Course slow.

(472) Lesions. Involvement of both lungs. Course slow. Condition poor.

(473) Lesions. Involvement of both lungs. Course slow. Condition bad.

(475) Lesions. Consolidation of right apex. Involvement of right lung and left apex. Course slow. Condition good.

(476) Lesions. Left apex consolidated, scattered lesions through left lung. Abscess of cervical nodes, incised four years ago. Condition good.

PULMONARY TUBERCULOSIS—ADULTS

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
477	Sputum	H.Type 1	Non-virulent	A.T.	23 yrs.	F.	?	Pulmonary Tuberculosis
478	"	"	"	M.C.	28 "	F.	1 yr.	"
480	"	H.Type 2a	"	M.C.	39 "	F.	1 "	"
481	"	H.Type 1	"	S.L.	34 "	F.	1 "	"
483	"	"	"	M.L.	88 "	F.	sev. yrs.	"
484	"	"	"	M.H.	30 "	F.	1 yr.	"
485	"	"	"	M.P.	43 "	F.	6 yrs.	"
486	"	"	"	L.M.	29 "	F.	3 "	"
487	"	"	"	L.J.	35 "	F.	5 mos.	"
499	"	"	"	W.A.	35 "	M.	6 "	"
500	"	H.Type 2a	"	?	Adult	?	?	"

(477) Lesions. Involvement of both upper lobes. Condition poor.

(478) Lesions. Scattered lesions of left lung. Course slow. Condition good.

(480) Lesions. Consolidation right lung. Condition good.

(481) Lesions. Both apices involved. Course slow. Condition poor.

(483) Lesions. Consolidated right lung. Course slow. Condition poor.

(484) Lesions. Involvement of right apex and left lung. Course rapid. Condition poor.

(485) Lesions. Involvement of left apex and right lung. Course slow. Condition poor. Axillary nodes removed five years ago.

(486) Lesions. Both apices involved. Condition fair.

(487) Lesions. Consolidation left apex. Course slow. Condition good.

(499) Lesions. Both apices involved.

(500) No details obtainable.

PULMONARY TUBERCULOSIS—CHILDREN

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
15	Sputum	H.Type 1	Non-virulent	E.B.	11 mos.	M.	1 mo.	Pulmonary Tuberculosis
50	"	"	"	J.M.	14 yrs.	M.	?	"
156	"	"	"	A.R.	1 yr.	F.	?	"
225	"	"	"	J.F.	15 yrs.	M.	6 mos.	"
277	"	"	"	?	6 "	?	5 "	"
406	"	"	"	E.S.	15 "	F.	11 "	"
409	"	"	"	H.G.	14 "	M.	1 yr.	"
416	"	"	"	J.T.	13 "	M.	1⅔ yrs.	"
471	"	"	"	H.M.	14 "	F.	7 mos.	"
479	"	"	"	J.L.	11 "	F.	2 yrs.	"

(15) History. No tuberculosis in family. Breast fed for four months. Then pasteurized milk (Straus). One month ago the child began to be feverish and to cough, especially in the morning. There was loss of weight.

Physical examination. A rachitic child. Chest expansion poor. Broncho-vesicular breathing over entire upper right lobe, with rales and dullness. Signs of cavity just above the nipple. The left lung shows a few scattered rales. Discharged two weeks later. Condition in lungs unchanged. Child much weaker.

(50) Lesions. Right and lower lobe and left base involved.

(156) History. Nursed for a period of time unstated, and then given cows' milk. Illness began with cough, nasal discharge, otitis media and gastro-intestinal disturbances. The cough increased in severity and the child lost weight. Physical examination, a cachectic child with enlargement of all the superficial lymph nodes. There was consolidation of the right apex, which gradually involved the entire upper lobe, and infiltration of the remainder of the right lung and all of the left. The spleen was palpable. The symptoms gradually increased in severity. Death occurred three months later. No autopsy.

(225) Lesions. Consolidation of entire right upper and part of middle lobe, scattered areas in left upper lobe. Progress acute.

(277) History. Measles about five months ago, followed by a cough, which has persisted until the present. There has been a loss in weight. Involvement of the right apex.

(406) Lesions. Second stage. Progressive.

(409) Lesions. Third stage. Improving.

(416) Lesions. Second stage. Improving.

(471) Lesions. Extensive involvement of left lung. Course rapid. Condition poor.

(479) Lesions. Consolidation of right upper lobe. Lumbar lordosis. Course slow. Condition good.

Note.—See Generalized Tuberculosis Children, Nos. 1, 20, 58A, 63, 66, 71, 98, 123, (164 & 165), 273, 279, 292, 388, 392, 435 for other pulmonary cultures.

TUBERCULOUS ADENITIS—ADULTS

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
31	Cervical Nodes	H.Type 1	Non-virulent	S.H.	20 yrs.	M.	?	Tuberculous Adenitis
185	"	"	"	A.J.	33 "	M.	6+ yrs.	"

(31) History. Three years ago lumps appeared in right side of neck, steady increase in size. Physical examination, negative except for enlarged nodes. Excision. Many of the nodes found broken down.

(185) History. Operated on six years ago for cervical adenitis on left side. Soon after the operation the nodes reappeared; two weeks ago he noticed enlargement of those on the right side. Physical examination shows a chain of enlarged nodes in the left sub-maxillary region, extending anteriorly almost to the median line. Several of these are as large as an almond, but most of them are smaller. On the right side, at the angle of the jaw, there is a node the size of a large almond. On both sides thre are enlarged nodes in the triangle of the neck as far down as the clavicle. The posterior cervical, axillary and inguinal nodes are all somewhat enlarged. All of the nodes mentioned are freely movable. The heart and lungs are normal.

Operation. Excision. The material examined is a cervical node the size of a filbert. On section this shows a very few white spots; otherwise it is hard and fibrous. The post-operative history was uneventful.

Pathologist's report, tuberculous adenitis.

TUBERCULOUS ADENITIS*—CHILDREN

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease	Diagnosis.
7	Cervical Nodes	H.Type 1	Non-virulent	F.F.	15 yrs.	F.	1 yr.	Tuberculous Adenitis
18	"	H.Type 2a	"	M.deF.	11 "	F.	1 yr. +	"
28	"	H.Type 1	"	C.S.	4½ "	F.	Sev. mos.	"
29	"	"	"	L.D.	12 "	F.	1 wk.?	"

(7) History. One year ago a swelling on the left side of the neck was lanced. The sinus healed in about a week, but the swelling remained, and has progressively enlarged. Examination shows on the left side a tonsillar node as large as a lemon and a sub-maxillary node of about the same size. In the posterior triangle the nodes are about the size of a pea. The right sub-parotid node is as large as a walnut, and on this side the sub-maxillary and the posterior cervical nodes are pea size. Excision. Nearly all the nodes were caseous throughout. Completely healed except for a few granulating areas, three months after operation. Pathological examination, caseous tuberculous nodes.

(18) History. Enlarged nodes were removed from the right side of the neck one year ago, including one below the angle of the jaw the size of an olive, and several in the deep cervical chain. At the time of operation the left sub-parotid was almond size, but it was not removed. One month ago the child had a sore throat, and it was noticed at that time the left cervical nodes were enlarged. Enlargement has been progressive from the time when it was first noticed. Examination shows the left tonsillar node enlarged, with a small abscess superficial and anterior to the sterno-mastoid muscle, the whole mass being about the size of a lemon. The lymph nodes in the posterior triangle are the size of lima beans and the sub-maxillary node is palpable. Excision. All the nodes are caseous. The wound was completely healed twenty-four days afterward. Pathological examination, caseous tuberculous nodes.

(28) History. A swelling in the neck was noticed several months ago. Examination shows a poorly nourished child with a grossly symmetrical swelling on each side of the neck, most marked beneath the angle of the jaw and over the upper part of the sterno-mastoid. The nodes which make up this tumor are partly matted together and are individually about the size of pigeon eggs. In the posterior triangle the lymph nodes are slightly enlarged and the axillary, inguinal and epitrochlear nodes are palpable. Excision of the right nodes, and one week later of the left nodes. Discharged, cured. Pathological examination, tuberculous nodes, almost completely caseous.

(29) History. Tonsils removed one year ago. A swelling in the neck was noticed one week ago, following a sore throat. Examination shows in the right sub-maxillary region a mass about the size of a lemon. The nodes in the tonsillar region are palpable and in the posterior triangle they are about the size of peas. The left sub-maxillary node is the size of a pigeon's egg, the tonsillar node of a small olive, and in the posterior triangle there are a few nodes slightly enlarged. Excision of the nodes on the right side. Discharged six weeks later, improved. Pathological examination, tuberculous nodes with very slight caseation.

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* Cervical, axillary, inguinal, etc.; those of a thoracic or abdominal cavity given elsewhere.

NOTE.—See Generalized Tuberculosis Children, No. 434 for other cultures from lymph nodes. Also Abdominal Tuberculosis Children, No. 155.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name	Age.	Sex.	Duration Disease.	Diagnosis.
67	Cervical Nodes	H Type 1	Non-virulent	E.H.	6 yrs.	F.	?	Tuberculous Adenitis
94	"	"	"	H.B.	8 "	M.	7 yrs.	"
95	"	"	"	M.Z.	9 "	F.	1 yr.	"
96	"	B Type 1	Virulent	B.B.	21 mos.	F.	8 mos.	"

(67) History. The lymph nodes on the right side of the neck have been enlarged for some time. The lesion was thought at first to be a simple inflammatory hyperplasia. Examination shows an olive-sized movable node at the angle of the jaw, and a palpable tonsillar node on the left side. Excision. Discharged a month after operation, cured. Pathological examination, many tubercles and a few small patches of caseation.

(94) History. The child has had nodular swellings on the left side of the neck since infancy. These were operated on nine months ago and the resulting wound healed in three weeks. The swellings have gradually increased in size. Examination shows the left tonsillar node to be about the size of a lemon. The nodes in the posterior triangle are palpable, as are the deep maxillary nodes. Below the angle of the jaw there is a small, superficial abscess. Excision. Discharged, cured, about two weeks and a half after operation. Pathological examination, caseous tuberculous nodes.

(95) History. The enlarged nodes were first noticed about one year ago, since which time they have been gradually increasing in size. Examination shows on the right side, enlarged nodes behind and below the maxillary angle, about the size of pigeons' eggs. The nodes beneath the sterno-mastoid and in the posterior triangle are palpable. On the left side the cervical, axillary, epitrochlear and inguinal nodes are enlarged. Excision of the nodes on the right side. Discharged six weeks later, cured. Pathological examination, caseous tuberculous nodes.

(96) History. Best surroundings, no house infection. Received milk of good quality which was not sterilized. About August 20, 1907, enlarged lymph nodes noticed on left side of neck; the temperature at this time varied from 102°-105° F. Has had more or less rise in temperature ever since. About December 15, the nodes on the right side were found to be enlarged. Lymph nodes of left side excised January 2, 1908. They were found to be much broken down and showed many caseous spots. The wound healed by January 15. On April 22 similar nodes excised from right side. Healing was slow and complicated by otitis media and bronchitis, completely healed by summer. No signs remain, the child being in good health.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
116	Cervical Nodes	H. Type 1	Non-virulent	C.F.	2 yrs.	M.	?	Tuberculous Adenitis
149	"	"	"	E.K.	6½ "	F.	1 yr.	"
150	"	"	"	J.C.	10 "	M.	?	"
161	Inguinal Nodes	"	"	G.R.	5 "	M.	5 mos.	"

(116) History. No history obtainable. Examination shows nodes about the size of an egg on the left side of the neck. The right tonsillar node is somewhat enlarged. Excision. Discharged, cured, six weeks later. A few small palpable nodes found about two months after operation. Pathological report, tuberculous nodes with but little caseation.

(149) History. One year ago a lymph node in the median line of the neck in the region of the larynx broke down, but soon healed. Two months later a node in the right sub-maxillary region opened spontaneously. Two months ago the left sub-maxillary node became involved. Examination shows large tuberculous ulcers in the sub-mental and sub-maxillary regions. The sub-mental nodes are partly sloughed away. The tonsillar nodes on both sides are enlarged, and the right sub-maxillary and sub-parotid nodes are greatly enlarged and caseous. The nodes in the posterior chain are the size of peas. Excision of nodes both sides of neck. Discharged one month later in good condition with the wound healed, but with a few nodes still palpable. Pathological examination, tuberculous nodes.

(150) History. Examination shows enlarged lymph nodes about the size of an olive on both sides of the neck. Excision of three nodes from the left side, varying in size from a filbert to a hickory nut. The tonsillar node was also removed. Discharged eight days later, cured. About six weeks later examination showed a few cervical nodes on the left side, and the axillary, epitrochlear and inguinal nodes palpable. Pathological examination, tuberculous nodes with but little caseation.

(161) History. In January, 1908, the lymph nodes in the left groin became swollen and broke down, leaving a persistent sinus. Excision June 5, 1908, of inguinal nodes the size of a hazel nut, all showing either complete caseation or caseating centers. Discharged three weeks after operation, cured.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
162	Cervical Nodes left side	H Type 1	Non-virulent	M.McC.	10 yrs.	F.	2 mos.	Tuberculous Adenitis
201	Cervical Nodes right side	"	"					
163	Cervical Nodes	B Type 1	Virulent	F.H.	3 "	M.	3 "	"
167	"	H Type 1	Non-virulent	L.S.	4½ "	M.	9 "	"
184	"	B Type 1	Virulent	J.T.	4 "	F.	1½ "	"

(162) History. Enlarged cervical nodes first noticed April, 1908, following a sore throat. Examination, May 26, shows a large bunch of nodes beneath and projecting in front of sterno-mastoid muscle on both sides in tonsillar region, the left showing the larger mass. There are numerous palpable nodes in the left posterior triangles, some large nodes the size of an olive along jugular and beneath angle of jaw, and a few small nodes in lower part of right posterior triangle. Excision of left nodes June 5, 1908, and of right nodes July 7, 1908. Wound in right side did not heal, and on August 31 was open inward as far as trachea and downward as far as mediastinum. Signs of cavity in apex developed; temperature 100°-103. Became gradually worse, and died September 23, 1908. No autopsy.

(163) History. Three months ago there was noticed a swelling in the neck, which is said not to have increased in size. Examination shows enlargement of the right tonsillar node to the size of a small lemon. It is freely movable. In the sub-maxillary region the nodes are about the size of peanuts, and in the posterior triangle they are enlarged to about the same size. Excision. Discharged, cured.

(167) History. Swelling in neck of nine months' duration. Examination shows on the left side a swelling in the tonsillar region about the size of a lemon, overlying which there is an area of reddened skin surrounding a small incision. The mass is composed of various-sized nodes, matted together. The posterior cervical and sub-maxillary nodes are palpable. Excision. Discharged, cured, about six weeks after operation. Pathological report, nodes contain miliary tubercles and areas of caseation.

(184) History. Swelling on the right side of the neck, first noticed early in May, 1908. Examination shows a fluctuating mass about the size of a pigeon's egg, overlying the middle of the jaw. The nodes in the posterior triangle are pea size. Excision June 19, 1908. Discharged about five weeks after operation, a few areas of granulation tissue remaining. Pathological report, chronic inflammation. Not tuberculous.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
201	See 162							
205	Cervical Nodes	H. Type 1	Non-virulent	G.T.	12 yrs.	M.	2½ yrs.	Tuberculous Adenitis
216	"	B. Type 1	Virulent	R.S.	9 "	M.	1½ "	"
217	"	"	"	A.A.	4 "	F.	?	"
247	"	"	"	C.L.	15½ "	F.	5 wks.	"
260	"	"	"	H.H.	15 mos.	M.	1 yr.	"

(205) History. Father and one brother dead of tuberculosis. Two and a half years ago a swelling in the neck opened spontaneously, and there have been other smaller abscesses since. The present swelling is of six months' duration. Examination shows the right tonsillar node to have attained the size of a hen's egg. The nodes in the anterior chain are olive size, while those in the posterior chain are shot size. On the left side the tonsillar node is the size of a small olive, and the anterior chain is enlarged, but the nodes are of smaller size than the tonsillar. The axillary nodes are pea size and the epitrochlear is palpable. Lymph nodes excised. Discharged twelve days later in good condition with wound healing. Pathologist's report, tuberculous nodes, largely caseous.

(216) History. Eighteen months ago the lymph nodes on the right side of the neck became enlarged, and seven months ago they began to increase rapidly in size. Examination shows a mass of nodes on the right side, behind the angle of the jaw, each one about the size of a hazel nut. The nodes in the posterior chain are the size of a walnut. Excision. Discharged, cured, seven days after operation. Pathological examination, tuberculous nodes, with many large and small tubercles and some caseation.

(217) History. Measles recently, since which there has been a persistent rhinitis. Examination shows hypertrophied tonsils, enlarged cervical nodes. Excision of lymph nodes. Discharged about three weeks after operation, cured. Pathological examination, advanced tuberculosis with caseation.

(247) History. Uncle and aunt died of tuberculosis before the patient was born. The enlarged lymph nodes were first noticed after a cold five weeks ago. Examination shows moderately enlarged nodes at the angle of the jaw on the right side. Excision. Discharged, cured.

(260) History. About eleven months ago the right side of the neck was lanced; the wound did not heal. Three months afterward another operation was done at the same site. This wound healed, but subsequently reopened. Since the time at which this last operation was done a new swelling on the same side has appeared, and has been gradually increasing in size. Operation. Excision of six or seven nodes in the sub-parotid region, varying in size from a small pea to a small chestnut. These nodes are all firm and readily decapsulated. Post-operative history uneventful.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
262	Cervical Nodes	B. Type 1	Virulent	L.O.	9 yrs.	F.	1 yr.	Tuberculous Adenitis
272	Inguinal Nodes	H. Type 1	Non-virulent	J.L.	6 "	M.	6 wks.	"
278	Cervical Nodes	"	"	C.C.	10 "	M.	1 yr.	"
375	"	"	"	J.G.	2½ "	M.	6 mos.	"
381	"	B. Type 1	Virulent	J.B.	5½ "	M.	10 "	"

(262) History. About a year ago, preceding by five weeks an attack of measles, a small swelling was discovered behind the angle of the jaw on the left side. This remained stationary in size until a month ago, when it began to grow larger. Examination shows, on the left side, a node about the size of a horse-chestnut at the angle of the jaw, and a few small nodes down the side of the neck. On the right side there are numerous enlarged nodes, the largest of which, situated at the angle of the jaw, is about the size of a hickory nut. Excision. Discharged about three weeks after operation cured. Pathological examination, advanced tuberculosis, with large and small tubercles and large areas of caseation.

(272) History. Six weeks ago the child cut his foot on a piece of glass. The wound healed, but opened again, and is now discharging pus. For a week he has had a painful swelling in the groin and some fever. Examination shows an incised wound healed, except for an area in the center. In the groin the lymph nodes vary in size from that of a pea to that of a hickory nut, and there is one node of the latter size just above Poupart's ligament. Excision of ulcer and nodes. Further course uneventful. Pathological examination, tuberculous ulcer. Tuberculous nodes, with many large and small tubercles, but with little caseation.

(278) History. Father died of tuberculosis. One year ago a lump appeared at the angle of the jaw. A month ago this was opened and a little pus escaped. Examination shows nodes varying in size from a pea to a pigeon's egg, beneath the symphysis menti and the border of the jaw. The axillary and inguinal nodes are palpable. Excision. Discharged eleven days after with the wound healed. Pathological examination, tuberculous nodes, largely caseous, with some calcification.

(375) History. Following measles, six months ago, the lymph nodes on the left side of the neck began to enlarge. One of them was incised two weeks after it was first noticed, and a sinus has persisted since that time. Examination shows the right sub-parotid node to be the size of a hickory nut, and enlargement of some of the nodes along the posterior border of the sterno-mastoid. On the left side there is a node of similar size at the angle of the jaw, and a chain extending downward as far as the clavicle. Excision. Discharged cured about three weeks after operation. Pathological examination, many tubercles and small areas of caseation.

(381) History. Sub-parotid nodes in right side of neck enlarged for ten months. Excision. Abscess, sub-maxillary, right side. Caseous nodes have many adhesions about them.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
393	Cervical Nodes	B. Type 1	Virulent	E.C.	4 yrs.	F.	2 yrs.	Tuberculous Adenitis
394	" (right side)	H. Type 1	Non-virulent	M.K.	7 "	F.	2 "	"
395	Cervical Nodes (left side)	"	"					
396	Cervical Nodes	"	"	H.A.	12 "	F.	1½ "	"
442	"	"	"	R.J.	12 "	F.	1 yr.	"

(393) History. There has been a swelling on the right side of the neck for the past two years, which, in the last six months has enlarged rapidly. There are many enlarged nodes on the right side and a subcutaneous abscess just in front of the sterno-mastoid muscle. On the left side there are several nodes at the angle of the jaw which have attained the size of a hazel nut. Excision. Discharged cured about two weeks later. Pathological examination, advanced tuberculosis.

(394) History. Following measles, two years ago, enlarged lymph nodes appeared in the neck. Those on the left side remained quiescent until five days ago, when they began to enlarge rapidly. The nodes on the right side have not enlarged since their discovery, two years ago. Examination shows the right sub-parotid and sub-maxillary nodes enlarged, and an enlarged node on the left side behind the angle of the jaw. Excision of nodes on both sides of the neck. Discharged cured a month afterward. Pathological examination, tuberculous nodes, with large areas of caseation.

(396) History. One and one-half years ago a small lump appeared behind the angle of the jaw. This was removed six months later. Others developed and were removed, and finally another one appeared in the neck and one in the cheek. Examination shows the right sub-parotid to be the size of a hickory nut, and the right sub-maxillary as large as a hazel nut. Both sub-mentals are enlarged. The left sub-maxillary is the size of a hickory nut, and in the left sub-parotid there is a sinus surrounded by keloid scar. Along the border of the sterno-mastoid muscle there is a hard, irregular mass. Excision. Discharged about three and one-half weeks after operation, healed. A few nodes still palpable. Pathological examination, tuberculous nodes, largely caseous.

(442) History. One year ago there was noticed a swelling in the neck. This has steadily increased in size. Examination shows, on the right side, an enlargement of the sub-parotid, sub-maxillary and anterior and posterior chains. These nodes vary in size from that of a pea to that of a hazel nut. The corresponding nodes on the left side are enlarged, but they have not reached the size of those on the right. In the groin there are a few nodes palpable. Excision of the nodes on the right side. Discharged two weeks after operation, healed.

GENERALIZED TUBERCULOSIS. ADULTS

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
308	Spleen	H.Type 1	Non-virulent	S.E.	23 yrs.	M.	4 mos.	Generalized Tuberculosis

(308) History. Family history is negative, and he has always been well and strong. On June 26, 1908, he had a chill followed by sweating; this recurred every night until very recently, when they became somewhat irregular in appearance, sometimes skipping a night. There has been a slight headache present most of the time, but no vomiting or joint pains. The night before admission, July 14, 1908, he was seized with a sharp shooting pain in the right side, in the area between the sixth and tenth ribs. This pain was relieved upon sitting up; breathing had no effect on it, and it was not made worse by coughing. In the right abdomen there has been present a very severe pain, which is aggravated by breathing and by coughing. The leucocyte count on admission is low, the differential count normal, and a blood culture is sterile. After admission there was a gradual decline in strength, the sputum became blood tinged, larger hæmoptyses appeared and death occurred October 27, 1908.

Autopsy. Anatomical diagnosis, general miliary tuberculosis involving liver, lungs, spleen, kidneys, peritoneum, intestine, lymph nodes and pleura.

Peritoneum smooth and glistening. The omentum is rolled into a thick shotty cord along the right border, and contains small white areas throughout. On the visceral peritoneum there are occasional conglomerated areas. The spleen is surrounded by adhesions. The right lung is more or less adherent to the diaphragm. Heart normal. Left lung almost entirely solidified with caseation of the lymph nodes at the hilum and a cavity in this region communicating with the large bronchi. The right lung contains small shotty areas throughout, and its lymph nodes are enlarged. The peritracheal nodes are large and cheesy. Stomach, duodenum and jejunum normal. The ileum contains two ulcers about the size of a five-cent piece, and occasional miliary tubercles in its mucosa. Colon normal. Liver surface dotted with numerous small white areas. At the hilum of the gall bladder there are a number of enlarged nodes. Spleen—a large number of enlarged caseous areas, some of which are conglomerate and all of which are cheesy in the center. Pancreas normal. Left kidney, small white areas in the cortex. Right kidney same as left.

GENERALIZED TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
336	Spleen	H.Type I	Non-virulent	H.G.	29 yrs.	M.	I yr.	Generalized Tuberculosis

(336) History. Previous history, negative except for smallpox in early youth. He has been in poor health for a year and unable to work for the past six months. During the year he has had a slight cough, with night sweats and loss of flesh. He has had also pain in the abdomen and has been constipated. Died.

Autopsy. Anatomical diagnosis, tuberculous enteritis and peritonitis, septic peritonitis, tuberculosis of lungs, liver, spleen, kidneys and pleura. The peritoneum is covered with small tubercles and the intestinal coils are glued together. There are about 150 c.c. of yellowish-green, foul fluid in the peritoneal cavity. The omentum is rolled up on the transverse colon and is very much thickened, sections of it showing large caseous areas. In the mesentery of the ileum and of the ascending colon there are large caseous masses. Heart negative. Left lung firmly adherent to pleura, diaphragm, pericardium and mediastinum. It is firm and slightly crepitant and shining through the pleura are small white areas. On section, the lung is seen to be studded with many small tubercles. Right lung, adherent to pleura on all sides, with the upper lobe, which contains an enormous number of small tubercles, practically consolidated. Peribronchial nodes not caseous. Stomach normal except for a few tubercles along the lesser curvature on the anterior surface. Pancreas, duodenum and jejunum normal. In the ileum there are ragged, shelled-out ulcers extending through the peritoneum, transverse in their long diameter. The cæcum is thickened and there is a large ulcer in the mucosa. Liver, tubercles on its surface and in its substance. Spleen, many tubercles on the surface, as well as in the substance. Both kidneys contain a moderate number of tubercles on their surfaces.

GENERALIZED TUBERCULOSIS—CHILDREN*

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
1	Sputum	H.Type 2a	Non virulent	A.R.	5 mos.	M.	2½ mos.	G Miliary Tuberculosis
45	Feces	"	"					
16	Mesenteric Node	H.Type 1	"	J.C.	1½ yrs.	M.	?	Generalized Tuberculosis including Meninges
19	Spleen	"	"					
20	Bronchial Node	"	"					
45	See No. 1							

(1) History. Mother died of tuberculosis. Breast fed at first, later Nestle's food. Illness began 2½ months ago, January 7, 1908, with cough. Gradual emaciation and temperature fluctuating around 98° and 99°, occasionally rising to 101°-102°. Died March 20, 1908.

Autopsy. Bronchial nodes enlarged on right side. One of these is softened. On left side these nodes contain cheesy tubercles, but are scarcely enlarged. Mediastinal nodes enlarged. Upper lobe of right lung has pleural adhesions over its posterior surface. These tear when the lung is removed and reveal a cavity 1½x1½ inches, containing greenish pus and cheesy material, and communicating with the main bronchus. What is left of the apex is in a condition of cheesy pneumonia, and there are many cheesy tubercles on the surface of the lobe. The anterior half of the upper lobe and the entire middle lobe are well aerated and studded with gray and yellow tubercles. The left apex contains an area of cheesy pneumonia anteriorly. There are many tubercles throughout the substance of both lobes. Heart normal. Spleen contains many very small tubercles. The liver is congested, and contains a few early tubercles. The colon shows about six small round tuberculous ulcers not involving the peritoneum. The solitary follicles of the small intestine and Peyer's patches are ulcerated, some of the ulcers being longitudinal and others transverse. There are a few young ulcers in the duodenum and many in the ileum which involve the peritoneal coat. The kidneys are congested and the capsule free. The left kidney contains one tubercle. The brain contains no tubercles. The pia is œdematous.

(16) History. Foundling child. Given out to board. Three other children given to the same woman died of tuberculosis. She had an adult boarder with tuberculosis who helped care for the children. Feeding consisted of condensed milk, oatmeal and milk. Details as to clinical course of disease not obtainable. Cause of death given as marasmus.

Autopsy. Anatomical diagnosis, tuberculosis of brain, lungs and liver, spleen and lymph nodes. Cervical nodes tuberculous. Lungs tuberculous. Mediastinal and bronchial nodes caseous. Liver, many tubercles. Spleen, many tubercles. Kidneys, negative. Small intestine, small tuberculous nodules at intervals. Large intestine, normal. Mesenteric nodes enlarged and caseous. Brain, increase of fluid and a few miliary tubercles along the vessels of the temporal lobes.

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* Case No. 382 is a primary intestinal tuberculosis. No. 122 is probably of the same type. These two cases follow in this series (see pages 52 and 47).

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
58 A	Sputum	H. Type 1	Non-virulent	J.C.	5 mos.	M.	3 wks.	Generalized Tuberculosis Including Meninges
58 B.	Cerebro-spinal Fluid	"	"					
62	Mesenteric node	B. Type 1	Virulent	M.B.	3½ mos.	F.	?	Generalized Tuberculosis

(58A) History. Breast fed. Illness began February 18, 1908, with irratibility, fever and vomiting. In a few days convulsions occurred. The head was retracted, respiration was irregular and the child was drowsy. The pupils were widely dilated and did not react to light, and there was marked internal strabismus. Kernig's sign was prominent. Lumbar puncture resulted in the withdrawal of 20 c.c. of clear fluid, in which tubercle bacilli could be demonstrated. Died March 8, 1909.

Autopsy. Anatomical diagnosis, acute generalized miliary tuberculosis, involving lungs, liver, spleen, lymph nodes, intestines and kidneys. Lungs, no pleurisy. Miliary tubercles studded over lobes of both lungs. Pea-sized cheesy nodule in right lower lobe posteriorly. Bronchial nodes, largest on right side, where one measures 1 inch in length and is softened. On the left side these nodes contain miliary tubercles. Heart, normal. Spleen, 4x2x2 inches, and closely studded with tubercles. Liver, miliary tubercles throughout. Stomach, normal. Kidneys, congested. Capsule, free. A few tubercles in cortex. Intestines, throughout duodenum and jejunum all the solitary follicles are enlarged and ulcerated; but in no case is the peritoneal coat involved. No ulcers in ileum or colon. Examination of brain not permitted.

(62) History. Fed on cow's milk. Entered hospital February 3, 1908, for repair of harelip and cleft palate. Physical examination was negative. On February 4, 1908, was given a routine Calmette test, which was positive. The physical examination was repeated, with negative findings as before, but tubercle bacilli were found in the sputum. After the operation the temperature rose and cough appeared, with rales in the lungs. In a few days consolidation appeared in the right upper lobe posteriorly, child grew steadily worse and death took place March 18, 1909.

Autopsy. Anatomical diagnosis, acute miliary tuberculosis of pleura, lungs, spleen, liver, intestine, lymph nodes and kidney, broncho-pneumonia. Lungs, no pleural adhesions. Tubercles along parietal pleura on both sides. Right lung, upper lobe completely solid from a recent broncho-pneumonia and a widespread involvement with tubercles. Two cheesy nodules, one near the center and one near the posterior border, each ¼ inch in diameter. Middle lobe well aerated and studded with tubercles. The lower lobe is broncho-pneumonic along the posterior border and closely studded with tubercles. Left lung, apex contains a small area of pneumonia, and tubercles are very numerous. The lower lobe contains an area of pneumonia at the posterior superior angle. The tubercles are smaller and less numerous than in the upper lobe. Bronchial nodes on the right side, the largest one is cheesy and softened, while the others contain only recent tubercles. The left are scarcely enlarged and do not show tubercles. The cellular tissue in mediastinum and the aorta contains numerous tubercles. Stomach normal. Duodenum normal. Jejunum contains three early ulcers in the solitary follicles. Ileum, one early ulcer in a Peyer's patch. Colon, normal. Mesenteric lymph nodes, slightly enlarged. Many of them contain miliary tubercles. Spleen, 3x1½ inches, with many gray tubercles on the surface and in the substance. Peritoneum, normal. Kidneys, congested, with numerous tubercles on surface and cortex.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
63	Lung	B. Type 1	Virulent	E.H.	1¼ yrs.	F.	?	Generalized Tuberculosis
69	Mesenteric Nodes	"	"					
66	Bronchial	"	"	M.I.	2⅔ "	F.	?	"
71	"	H. Type 1	Non-virulent	W.G.	7½ mos.	M.	2 wks.	" including Meninges

(63) History. No details obtainable. Foundling child given out to board. Milk fed.

Autopsy. Anatomical diagnosis, empyema, tuberculosis of lungs, bronchial nodes, mesenteric nodes and intestines. Pleura, right, large empyema involving entire cavity; left, negative. Lungs, right, masses of breaking-down caseating pneumonia in both lobes; left, small, scattered tubercles. Bronchial nodes enlarged and caseous. Heart, liver and kidneys, negative. Spleen, many tubercles. Small intestine, several tuberculous ulcers. Large intestine, negative. Mesenteric nodes, enlarged and cheesy.

(66) History. Foundling child. Given out to board. Died of scarlet fever and measles. No further details obtainable.

Autopsy. Anatomical diagnosis, tuberculosis of cervical nodes, broncho-pneumonia, fatty liver, tuberculosis of bronchial and mesenteric nodes, ulcerative laryngitis. Tongue and larynx ulcerated. Cervical nodes, much enlarged. Lungs, pleura normal. Posterior half of both lobes consolidated. Right middle lobe anteriorly studded with miliary tubercles. Mediastinal nodes enlarged. One very large caseous node at bifurcation of trachea. Heart, negative. Liver, fatty. Kidneys, negative. Small intestine, Peyer's patches congested. Large intestine, coat thickened and congested. Mesenteric nodes enlarged and cheesy.

(71) History. Breast fed. Illness began May 9, 1908, with vomiting. The child soon became prostrated and feverish, and convulsions occurred. There was no cough. The child grew stuporous, Kernig's sign was positive and rigidity of the extremities and of the neck appeared, without retraction of the latter. Lumbar puncture resulted in the withdrawal of 40 c.c. of clear fluid, in which tubercle bacilli were found. The coma gradually deepened and death supervened May 22, 1908.

Autopsy. Anatomical diagnosis, tubercular meningitis, acute miliary tuberculosis of lungs, liver, spleen and lymph nodes. Brain, tubercles are very numerous along the blood vessels of the internal and upper surfaces of both hemispheres. The tubercles are very small and recent. At the base the pia in the interpeduncular space is very cloudy and is thickened and studded with many tubercles. Tubercles are numerous over the upper surface of the cerebellum. The lateral ventricles are not dilated. Heart, normal. Lungs, no pleurisy, pneumonia at the right apex. There are a few recent tubercles in all the lobes, but no cheesy nodules are present. Bronchial lymph nodes, on the right side two are 1 inch in diameter and show central softening; on the left side they are not enlarged, nor do the mediastinal nodes show any enlargement. Spleen, 3x1½x1 inches. It is closely studded with miliary tubercles, a few of which are yellow and cheesy. Liver, tubercles scattered throughout its substance. It is not enlarged. Stomach, normal. Intestines, no ulcers. Peyer's patches, as well as the solitary follicles, are slightly enlarged. Mesenteric nodes somewhat enlarged, but without tubercles. Kidneys, pancreas and peritoneum, normal.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
74	Cerebro-spinal Fluid	H. Type I	Non-virulent	M. Z.	19 mos.	F.	I mo.	Generalized Tuberculosis Including Meninges
97	Liver	"	"	M.McD.	6 mos.	F.	?	Generalized Tuberculosis
98	Lung	"	"					
99	Spleen	"	"					
100	Mesenteric Nodes	" "	" "					

(74) History. Breast fed 17 months, then table food. Measles a month ago, followed by a cough, which has persisted until the present time (April 4, 1908). For four days has been vomiting. Is prostrated and moans constantly. Lymph nodes palpable in the neck and groin. Internal strabismus and rigidity of the neck soon developed, and the child became gradually unconscious, screaming, however, if disturbed. Lumbar puncture resulted in the withdrawal of 40 c.c. of fluid, in which tubercle bacilli were found. Died April 14, 1908.

Autopsy. Anatomical diagnosis, general miliary tuberculosis, involving the brain, pericardium, pleura, omentum, lymph nodes, lungs, liver, spleen, pancreas, intestines, kidneys and meninges. Bronchial nodes, one on the right side is I inch long and contains cheesy tubercles. On the left side many are cheesy but not soft. None are larger than ⅜ inch. Lungs, no adhesions. Miliary tubercles on both visceral and parietal layers of pleura; left, a cheesy area ¾x¾ inch in apex. Strip of atelectasis along posterior border of lower lobe. Miliary tubercles in large numbers. Right, miliary tubercles in large numbers; no consolidation. Mediastinal nodes, all enlarged and cheesy. Pericardium shows a crop of miliary tubercles close to a tubercular mediastinal node. Miliary tubercles around origin of pulmonary artery, aorta and base of pericardial sac. Stomach, normal. Intestine, some cheesy solitary nodules throughout jejunum and ileum. One Peyer's patch just above the ileocæcal valve contains an early tuberculous ulcer. Mesenteric lymph nodes, slightly enlarged, containing cheesy tubercles. Pancreas, small group of cheesy tubercles in head. Spleen, 3½x2xI inches. Very closely studded with miliary tubercles, many of which are cheesy and measure I-16 inch in diameter. Liver, closely studded with miliary tubercles. Omentum, studded with miliary tubercles. Parietal peritoneum, no tubercles. One ounce of clear fluid in the peritoneal cavity. Kidneys, 6 to 10 tubercles in the cortex. Brain, many miliary tubercles along the vessels over the cortex of both hemispheres. At the base there is an exudation of fibrin and pus. The ventricles are distended.

(97) History. No details obtainable.
Autopsy. No details obtainable.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
122	Mesenteric Node	B. Type I	Virulent	F.A.	3⅚ yrs.	M.	?	Generalized Tuberculosis
123	Lung	"	"					
148	Cerebro-spinal Fluid	H. Type I	Non-virulent	J. VanH.	1½ "	M.	3 wks.	Generalized Tuberculosis Including Meninges

(122) Foundling child, boarded out to different nurses. No definite history of feeding. As far as ascertainable received bottled or store milk which was scalded, according to two of the nurses. At six months of age was in poor health, but later improved till final illness. Measles and broncho-pneumonia, with purulent otitis media, terminating in death. Duration two months.

Autopsy. Anatomical diagnosis, broncho-pneumonia, tuberculosis of mesenteric nodes. (No statement as to whether broncho-pneumonia was tuberculous or not.)

(148) History. Breast fed until one month ago, then table food. Illness began three weeks ago with projectile vomiting. After three days fever appeared, with pain on moving. The child was drowsy but conscious. Later rigidity of extremities and neck appeared, followed by convulsions. The pupils were dilated and reacted sluggishly. Axillary and inguinal nodes just palpable. Lumbar puncture resulted in the withdrawal of 90 c.c. of clear fluid in which tubercle bacilli were found. Died May 28, 1909.

Autopsy. Anatomical diagnosis, acute miliary tuberculosis, involving lungs, liver, spleen and lymph nodes, broncho-pneumonia, fatty liver. Heart, normal. Lungs, no pleurisy. Small wedge-shaped area of tuberculosis in right apex, as well as in the posterior portion of the middle lobe. Recent miliary tubercles over and throughout all the lobes, but most numerous in the right middle lobe. Bronchial nodes, on right side, two nodes, 1 inch long, cheesy and softened; on the left side the nodes are small and red. Anterior mediastinal nodes slightly enlarged and contain tubercles. Stomach, normal. Liver, moderately fatty, with many tubercles throughout its substance. Spleen, 3 inches long, firm and studded with miliary tubercles, several of which are cheesy. Kidneys, congested. Intestine, solitary follicles enlarged, especially in the cecum. The mucosa of the colon is congested. The ileum has a congested mucosa, with enlarged solitary follicles, but no ulcers. Mesenteric nodes, small. A few contain cheesy tubercles. Brain, not examined.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
164	Lung	H.Type 1	Non-virulent	I.B.	5½ yrs.	F.	1½ yrs.	Generalized Tuberculosis
165	Bronchial Node	"	"					
273	Lung	"	"	J.K.	1 yr.	M.	7 wks.	"
274	Spleen	"	"					

(164) History. Operated on one and one-half years ago for cervical adenitis. Sinus still present. Measles two months ago, complicated by broncho-pneumonia. Ankylosis of right shoulder joint.

Autopsy. Anatomical diagnosis, unresolved broncho-pneumonia; pulmonary tuberculosis; tuberculosis of bronchial and mesenteric nodes; tuberculous ulceration of intestines.

Cervical nodes, enlarged and cheesy; sinus present. Pleura thickened and adherent. Lungs—Right, broncho-pneumonic consolidation, cheesy, with pus exuding from bronchioles, cavity size of walnut in apex. Left, consolidation in both upper and lower lobes. Mediastinal and bronchial nodes enlarged, cheesy and tuberculous. Heart, liver, kidneys, spleen normal. Small intestine, congestion in patches. Large intestine, small ulcerations, apparently tuberculous. Mesenteric nodes enlarged and tuberculous. Abscess of left hip, ankylosis of right shoulder, both tuberculous in origin.

(273) History. Breast fed until one week ago. Five weeks ago, about August 9, 1908, diarrhœa began and later vomiting appeared. Gastro-enteritis continued and child died September 30, 1908.

Autopsy. Anatomical diagnosis, tuberculosis of bronchial, tracheal and cervical lymph nodes, miliary tuberculosis of lungs and spleen, gastro-enteritis, parenchymatous nephritis, undescended testicle, fatty degeneration of heart, liver and kidneys. Peritoneum, normal. Mesenteric nodes, enlarged and show on section, medullary swelling. Pleura and pericardium, normal. Heart, fatty degeneration. Lungs, do not collapse. Numerous areas of interstitial emphysema. On section they are anemic and markedly edematous. Pleura contains a moderate number of miliary tubercles. Miliary tubercles found upon sectioning the lung. Bronchial nodes, right, normal; left, extensive tuberculous involvement. The lymph nodes at the bifurcation of the trachea are also extensively involved. In the neighborhood of these nodes the lungs contain numerous miliary tubercles. Lower cervical nodes also involved. Spleen, enlarged and congested, with many miliary tubercles throughout its substance. Kidneys, capsule strips easily. Cortex swollen and marking obliterated. Pyramids slightly congested. Liver, fatty. No tubercles. Stomach, normal. Lower part of ileum and the large intestine contain altered blood. Mucosa of entire gastro-intestinal tract thickened and congested. Ileum and large intestine show numerous small hemorrhages and superficial erosions. Peyer's patches, slightly swollen. No evidences of tuberculosis. Pancreas normal. Brain could not be examined.

Histological examination. Kidney, extensive fatty degeneration. No tubercles. Liver, extensive fatty degeneration and infiltration. No tubercles. Spleen, moderate number of epitheloid tubercles. Mesenteric nodes, inflammatory hyperplasia. No tuberculosis.

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
276	Mesenteric Nodes	H. Type 1	Non-virulent	C.S.	14 mos.	M.	2 wks.	Generalized Tuberculosis

(276) History. Heated milk, then house diet from eight months old. No breast feeding. Two weeks ago, September 22, 1908, began to cough and to be feverish. In November adult in family found to have bad pulmonary tuberculosis. Temperature fluctuated from 99° to 104°. Signs in lungs grew gradually more marked, the child grew progressively weaker and finally died October 6, 1908.

Autopsy. Anatomical diagnosis, general tuberculosis, involving pleura, lungs, lymph nodes, spleen, liver, peritoneum and intestine. Peritoneum, contains about an ounce of dark, bile-stained fluid. Parietal peritoneum in region of liver shows a number of cheesy tubercles, from pin-point size to ⅛ inch in diameter. The omentum is closely studded with miliary tubercles. The lower surface of the diaphragm is adherent to the liver, and contains many yellow tubercles. Pleura, adhesions on right side, but not on left. Visceral and parietal pleura contain many tubercles. Lungs—Left, an exudate of recent thin fibrino-pus is found between the lobes and extends over the surface of the upper lobe, tubercles over the entire lung. Apex solid with broncho-pneumonia, as is also more than half of the lower lobe. Right, lower lobe contains scattered areas of broncho-pneumonia. The middle lobe is well aerated, and closely studded with miliary tubercles. The upper lobe shows an apex solid from broncho-pneumonia, while the anterior portion of the lung contains a cheesy mass 2x2 inches in diameter on the surface, and within a cavity 1½x2 inches in dimension. The cavity contains thin grumous material, and communicates with a large bronchus. Bronchial lymph nodes, very much enlarged. The largest on the right side is 1 inch in length and cheesy. None are softened. The mediastinal lymph nodes are all enlarged. At the level of the right clavicle, two lymph nodes are cheesy. Cervical nodes, slightly enlarged; no tubercles. Heart and stomach, normal. Spleen, much enlarged, with miliary tubercles on the surface and several cheesy tubercles in the substance. Liver, enlarged and studded with miliary tubercles, several of which are cheesy. There is a cheesy lymph node in the long fissure. Intestines, no ulcers in colon, but several in the ileum, just above the valve. These, of which there are about six, including a few in the jejunum, extend to the peritoneal coat. Mesenteric nodes, all enlarged and most of them cheesy. Pancreas, normal. Kidneys, soft and red, with the capsule free. There is a large number of tubercles in the capsule and in the cortex, several large cheesy areas in the medulla. Retroperitoneal nodes, cheesy.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
279	Bronchial Nodes	H.Type 1	Non-virulent	J.F.	1 yr.	M.	2 mos.	Generalized Tuberculosis
291	Mesenteric Nodes	"	"	C.F.	8 mos.	M.	?	"
292	Bronchial Nodes	"	"					

(279) History. Mother has pulmonary tuberculosis. Breast fed for eight months. Six weeks ago, August 1, 1908, began to cough, and since then has coughed continually. Sleeps well. Temperature range while in hospital, from 98° in the morning to 101° in the evening. Died October 4, 1908.

Autopsy. Anatomical diagnosis, tuberculosis of lungs, lymph nodes, spleen, liver, pleura, peritoneum, kidney and intestine. Lungs, no pleural adhesions except between right middle and upper lobes, where they are fairly well organized. Both lungs closely studded with gray miliary tubercles. The right middle lobe, which is the only consolidated portion, is completely solidified, and shows in its central portion a cavity 1 inch long and ¼ inch wide, containing red grumous material, and showing yellow tubercles in its walls. The blood vessels in the wall are much congested, so that the whole cavity has a red instead of a yellow color. There are very small scattered areas of pneumonia in the right upper lobe, and many of the tubercles in the substance of the lobe are conglomerated and cheesy. Congestion is very marked. Bronchial lymph nodes, the largest on the right side measures 1 inch in length and is completely cheesy. On the left side they are small and contain only tubercles. Mediastinal and deep cervical lymph nodes, all of these are enlarged, and some are cheesy. Trachea and larynx, congested. Pericardium, normal. Heart, normal. Foramen ovale closed. Thymus, normal. Spleen, slightly enlarged, firm and closely studded with gray tubercles. Liver, deeply congested, showing gray tubercles on the surface and around the bile ducts on section. Diaphragm, on the right side there are many tubercles, on both pleural and peritoneal surfaces. The remainder of the peritoneum is normal. Pancreas and suprarenals, normal. Kidneys, congested. Tubercles in boundary zone. Left kidney contains a yellow, adherent mass in the pelvis. Stomach normal. Duodenum, normal. Jejunum, several small round tuberculous ulcers, not involving the peritoneum. Ileum, about eight tuberculous ulcers in the solitary follicles outside of Peyer's patches. Cecum, a number of small round ulcers which are continued into the colon, and even into the rectum. In no case do they extend into the peritoneum. The mucosa between the ulcers is congested and the solitary follicles are enlarged. Mesenteric lymph nodes, all enlarged. The majority of them contain cheesy tuberculous areas, but no one of them is completely cheesy.

(291) No history obtainable, except that the child died of generalized tuberculosis. Foundling child, given out to board.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
377	Mesenteric Nodes	H. Type 4	Non-virulent	L.P.	3 mos.	M.	?	Generalized Tuberculosis

(377) History. Mother died of tuberculosis when baby was ten days old. Fed on cows' milk in various modifications. Appetite never good. On October 24, 1908, when three months old, showed no abnormal physical signs but weighed only five pounds three ounces. (Birth weight, five pounds.) A week later developed physical signs in lungs. Temperature range very irregular, from 98° to 103°. Died November 10, 1908.

Autopsy. Anatomical diagnosis, acute tuberculosis of pleura, lungs, spleen, liver, pancreas, lymph nodes and intestine. Lungs, pleural adhesions between left upper lobe and chest wall, organized. Entire left upper lobe consolidated. On section it shows cheesy pneumonia. The whole apex is softened, making a fluctuating mass, which has not yet become a cavity. The left lower lobe containing some broncho-pneumonia. The entire right lung is well aerated and closely studded with miliary tubercles. Bronchial lymph nodes, two on the right side measure one-half inch in length and encroach on the œsophagus. On the left side none are larger than one-fourth inch, but all contain cheesy areas. Thymus normal, but surrounded by cheesy mediastinal lymph nodes. Heart normal. Foramen ovale closed. Stomach normal. Kidneys and suprarenals normal. Pancreas, cheesy tubercles on anterior surface, near upper border. Spleen enlarged, contains a number of cheesy tubercles one-sixteenth inch in diameter. Liver deeply congested, contains about 20 tubercles one-sixteenth inch in diameter. Duodenum normal. Jejunum, six round typical tuberculous ulcers, none of which involve the peritoneal coat. Ileum, nine small round ulcers, two within a Peyer's patch. Mucous membrane between lymph follicles pale, but otherwise show no change. Large intestine, mucosa markedly congested. There are no ulcers, but the walls are slightly thickened and the solitary follicles are all enlarged. Mesenteric lymph nodes almost all cheesy and enlarged. None are more than one-fourth inch in diameter.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
382	Mesenteric Nodes	H.Type 1	Non-virulent	F.B.	4⅙ yrs.	F.	2½ mos.	Generalized Tuberculosis

(382) History. Mother coughed for 13 months before her death, lost weight, and expectorated, but there were no hemoptyses. Breast fed for two months; then received condensed milk for a year. Measles five months ago, April, 1908; has lost weight since. The present illness began eight weeks ago with weakness, loss of appetite, vomiting, irregular stools and evening temperature. The abdomen has been swollen for the past six weeks. There is pain in the abdomen occasionally during the day. The child is up and around, but stupid. Temperature range from 98° to 102.6° with marked remissions. Leucocyte count 15,300 October 29, 1908, operation. Cœliotomy. Several small tubercular nodules on the omentum and on the bladder and several small caseous masses in the mesentery. Oxygen allowed to flow in. Died December 17.

Autopsy. Anatomical diagnosis, primary intestinal tuberculosis. Chronic tubercular peritonitis, atelectasis, acute miliary tuberculosis of the liver, spleen and bronchial lymph nodes, chronic ulcerative tuberculous entero-colitis, cheesy tuberculosis of mesenteric lymph nodes Lungs, no pleurisy; no pneumonia. There is an area of atelectasis in the right upper lobe extending throughout the whole lower half. Bronchial lymph nodes, the largest is three-eighths inch in diameter and contains only young tubercles. None are cheesy. They are somewhat pigmented. Liver moderately congested with a mass of miliary tubercles over the right lobe, where it is adherent to the abdominal wall. There are very few tubercles in the liver substance. Spleen, 4 x 2 inches. Firm and studded with early miliary tubercles. Perisplenitis. Stomach, normal. Suprarenals and pancreas normal. Kidneys, acutely congested. No tubercles. Peritoneum, no fluid. Both parietal and visceral surfaces are studded with yellow tubercles varying from 1 to 6 m.m. in diameter. The adhesions between the intestinal coils are not marked, except over the cecum and sigmoid flexure, where they are too dense and thick to be readily separated. Over the entire small intestine there are only small tubercles and recent fibrin. The cecum and appendix are bound down by a dense inflammatory mass in which large, cheesy lymph nodes are embedded. On separating this mass and opening into the ileum, just above the cecum, a perforated ulcer is found in the lowest Peyer's patch. Another such perforation is found a little higher up. The wall of the cecum is greatly thickened and the mucosa is covered with large ulcers. The rectal wall is less thickened. The upper ileum is of normal thickness. The mass of mesenteric lymph nodes is in two halves: one adherent to the cecum and measuring 6 x 4 cm.; the other adherent to the signoid flexure and measuring 5 x 4 cm. Both are hard and composed of single nodes, 0.5 to 2 cm. long. All these nodes are cheesy, but not softened. The appendix is not perforated.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
388	Bronchial Nodes	H.Type 1	Non-virulent	J.A.	11½mos	M.	?	Generalized Tuberculosis
389	Mesenteric Nodes	H.Type 2a	"					
392	Bronchial Nodes	H.Type 1	Non-virulent	R.P.	2¼ yrs.	M.	?	"

(388) History. Bottle fed—cows' milk. Gastro-enteritis, with loss in weight. Cervical nodes palpable. Temperature range 98.8° to 102°. Cough and dyspnœa. Died.

Autopsy. Anatomical diagnosis, tuberculosis of pleura, lungs, lymph nodes, spleen, liver, peritoneum, intestine. Pleura, tubercles scattered over parietal layer. Lungs, left lung, no consolidation; numerous pinhead tubercles scattered through its substance. Right lung shows caseous pneumonia in its upper lobe, with a cavity at the apex containing cheesy material and measuring about 1x½ inches. This region is especially adherent to the chest wall. Both middle and lower lobes show areas of caseous pneumonia. Small tubercles are found over the entire surface of the lung. Bronchial lymph nodes, on the right side, are very large and caseous; on the left side they are swollen and contain small tubercles. Thymus, small. Liver, normal substance, with miliary tubercles scattered over the surface. Spleen measures 2x1½ inches. It is friable and red, with many tubercles on its surface and in its substance. One tubercle measures 2 m.m. Heart normal. Stomach, normal. Duodenum and jejunum, normal. In the lower fourth of the ileum there are four small ulcers, with an area of congestion surrounding them. Large intestine, the solitary follicles are swollen throughout, and in six there is definite ulceration. There is no special thickening of the walls. Mesenteric lymph nodes, all enlarged, many of them showing small areas of caseation. Suprarenals, kidneys and pancreas normal. Retroperitoneal lymph nodes enlarged, two showing definite caseation.

(392) History. Breast fed until the 15th month. Two weeks ago, November 2, 1908, began to have cough and dyspnœa. Loss in weight. Cervical nodes, palpable. Abdomen, distended. Cervical Potts Disease. Died November 17, 1908.

Autopsy. Anatomical diagnosis, caries of spine, miliary tuberculosis of lungs, liver, spleen and lymph nodes. Tuberculous ulceration of intestine. Atelectasis. Heart normal. Foramen ovale closed. Lungs, no pleurisy. Miliary tubercles numerous in both lungs. No cheesy material and no pneumonia. Area of atelectasis in right lower lobe. Bronchial nodes, largest on right side, where one is three-fourths inch in length and cheesy and dry on section. The others contain small tubercles only. Spleen, normal size with many tubercles in its substance. Liver, normal size. Congested with a few tubercles in its substance. Stomach, normal. Jejunum and ileum contain about six round typically tuberculous ulcers, which do not involve the peritoneum. Colon, mucosa congested. No ulcers. Mesenteric nodes all enlarged. A few contain cheesy tubercles. Suprarenals, kidneys and pancreas normal. Vertebral column, there is an abscess containing cheesy material, in front of the bodies of the fourth to eighth dorsal vertebræ. Denuded bone is encountered on incising this. The body of the seventh dorsal vertebra is almost entirely destroyed, while the fifth and sixth are not so extensively involved.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
434	Inguinal Nodes	H.Type 1	Non-virulent	A.H.	11 mos.	M.	6 wks.	Generalized Tuberculosis
435	Bronchial Nodes	"	"					

(434) History. An aunt whom the child frequently visited died eight months ago of an active pulmonary tuberculosis. Breast fed. About three weeks ago, October 15, 1908, the mother noticed that the abdomen was swollen and painful on pressure. There was loss in weight. Lymph nodes in groin and in neck palpable. Temperature range within 1½° of normal. The abdomen remained distinctly distended throughout the illness and death occurred on November 23, 1908.

Autopsy. Lungs very firm, adhesions between left lung and chest wall. Left lung, upper lobe almost completely solid, showing on section a broncho-pneumonia with a calcareous mass in the center. Lower lobe and lingula are well aerated. Right lung pale. No pleurisy. No pneumonia. Bronchial lymph nodes all enlarged. Those on the left side are completely cheesy; on the right side they are enlarged but not cheesy. Mediastinal nodes much enlarged and some of them cheesy. Pericardium, adherent to the left pleura. Heart normal. Foramen ovale closed. Abdomen, liver very adherent to diaphragm and to abdominal wall. Glisson's capsule is markedly thickened, and in it are cheesy tubercles varying in size from a pinhead to three-eighths inch. Tubercles are also very marked along the round ligament and on the gall bladder. The abdominal wall is four times its normal thickness, owing to œdema and the formation of innumerable tubercles.

TUBERCULAR MENINGITIS*—CHILDREN

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
2	Sputum	H.Type 1	Non-virulent	M.L.	5 mos.	F.	1½ mos.	Tubercular Meningitis
4	Cerebro-spinal Fluid	"	"					
61	"	"	"	M.S.	10 mos.	M.	5 wks.	"
72	"	"	"	W.B.	15 "	M.	7 days	"

(2) History. Breast fed. No tuberculosis in the house. The illness began December 15, 1907, with fretfulness and crying. Retraction of the head, clenching of the hands and strabismus began January 1, 1908. The child gradually became stupid and lay quiet, screaming, however, when the head was touched. Physical examination showed equally dilated pupils, which reacted to light, and marked internal strabismus. Lung, heart and abdomen negative. Head retracted, knee jerk much exaggerated. Thirty c.c. of bloody fluid were withdrawn by lumbar puncture February 2, 1908, and the following day 50 c.c. of turbid fluid, which contained tubercle bacilli. The child now began to cough and tubercle bacilli were demonstrated in the sputum. Convulsions appeared, followed by coma, and death on February 8, 1908. Temperature range 98° to 101°. No autopsy.

(61) History. Breast fed. Early in February the child began to cough and to have a high fever. Vomiting appeared, followed after an interval of a few days by convulsions. Physical examination showed the child to be drowsy but conscious, with marked internal strabismus. Lungs, many rales; no dullness. Heart a little irregular. Abdomen, negative. Marked general rigidity. Kernig's sign present. Lumbar puncture; withdrew 60 c.c. of clear fluid, which contained tubercle bacilli. Death occurred, preceded by coma, on March 18, 1908. Temperature range 100° to 104°. No autopsy.

(72) History. Has been growing drowsy since April 3d. Rapidly became comatose and hemiplegic. Retraction of neck, and positive Kernig's sign. Lumbar puncture; withdrew 35 c.c. of clear fluid, in which no tubercle bacilli could be found. Died April 11, 1908. No autopsy.

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* The cases are classified as simply tubercular meningitis in the absence of data as to how generalized a tuberculosis was present.

NOTE.—See Generalized Tuberculosis, Children, Nos. 58B, 74, 148 for other cerebro-spinal cultures.

TUBERCULAR MENINGITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
306	Cerebro-spinal Fluid	H.Type 1	Non-virulent	D.K.	7 yrs.	M.	1 mo.	Tubercular Meningitis
443	"	"	"	I.S.	11 mos.	F.	7 days	"

(306) History. Tubercular family history and history of contact both denied. Breast fed for 16 months; this feeding, however, having been supplemented by milk from a single cow. Measles at four years, no sequelæ. Two or three attacks of swollen lymph nodes, each one lasting for a few days, and generally involving the cervical nodes. Once, however, those in the axilla were concerned. Ten days before admission to the hospital he began to have headache, anorexia and vomiting, with abdominal pain and loss of flesh. The headache was worse at night. There were neither projectile vomiting nor sudden sharp outcries. Upon admission, October 12, 1908, the right pupil was larger than the left. Kernig's sign was marked, and there was tenderness over the cervical and upper dorsal vertebræ, with tension of the posterior muscles of the neck. The lungs were negative. About 10 c.c. of clear fluid was aspirated from the cerebro-spinal canal. After admission the child had all the signs and symptoms of cerebro-spinal meningitis, and died November 3, 1908. No autopsy.

(443) History. Breast fed. Illness began December 11, 1908, with drowsiness. When awake the child was very restless. Vomiting and constipation were present. At first there were no eye symptoms and no convulsions Examination, lymph nodes of neck and groin palpable. The skin is almost cyanotic and tache cerebrale is present. The pupils are irregular in reaction and unequal in size. The arms, legs and neck are rigid, but there is no opisthotonos. Respiration is irregular and at times shallow and rapid. At other times it is slow and deep. A few rales are heard in the region of the right nipple. The heart is normal and its action good. The abdomen is negative. Fifteen c.c. of clear fluid, containing tubercle bacilli, were obtained by lumbar puncture. The child grew progressively worse. Convulsions supervened, followed by coma and death, December 18, 1908. Temperature range 98° to 102.4°. No autopsy.

ABDOMINAL TUBERCULOSIS—CHILDREN

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
109*	Mesenteric Node	B. Type 1	Virulent	Baby W.	23 mos.	?	4 mos.	Tubercular Peritonitis
155	Cervical Node	H. Type 1	Non-virulent	M.L.	6½ yrs.	F.	3¼ yrs.	Tuberculosis of Cervicals Axillary and Mesenteric Nodes
604	Retrocecal Node	"	"				4⅙ yrs.	
433	Appendicular Node	B. Type 1	Virulent	R.D.	8 yrs.	F.	?	Tuberculosis of Mesenteric Nodes (of appendix)

(109) *History. Breast and certified milk for 18 months, upon which the baby thrived. The food was then changed and ordinary milk substituted. Shortly after this the child began to fail and developed symptoms of tubercular peritonitis. No tuberculosis suspected elsewhere. A cœliotomy was performed; miliary aubercles were found, extensively involving the peritoneum, and there was a great mass of omentum in the region of the ileo-cœcal valve. The child did not rally from the operation. Died soon afterwards. No autopsy.

(155) History. Mother tuberculous. In February, 1905, swelling noticed in left side of neck and an abscess formed. These nodes were excised April, 1907. The child was re-admitted to the hospital, February, 1908, and enlarged nodes were found in both sides of the neck, varying in size from size of shot to size of olive. In the right axilla a fluctuating mass present, the size of an orange. This axillary mass was removed. Later, June, 1908, the nodes from the right side of the neck were excised (155), the tonsillar node was the size of a lemon, the remaining nodes varying in size from pea to olive size. All showed tubercles and caseation. While under the anæsthetic, the mesenteric nodes were found palpable. In February, 1909, pain developed in the right groin. On examination, few discrete nodes palpable in cervical region. Abdomen rigid and mass felt in lower right quadrant. A laparotomy was performed. Some free peritoneal fluid present and a large retrocecal abscess and mass of caseous broken down nodes. These were removed (604). No evidence of involvement of lungs. Discharged April 21, 1909, improved, sinus still discharging.

(433) History. Three years ago she suffered an acute attack of appendicitis which lasted two or three days. A similar attack began a week ago, ushered in by pain and vomiting. The attack was not very severe, and there were no urinary symptoms. It lasted for about a week. Examination before operation was entirely negative. Appendectomy. Several nodes in the mesentery of the appendix, each about the size of a bean, were excised. Discharged, cured, one month after operation. Pathological examination, advanced tuberculosis of lymph nodes. Chronic appendicitis. No tuberculosis of the appendix.

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* The milk from the dealer who supplied the milk for this child was subsequently tested and tubercle bacilli were isolated.

Note.—See "Generalized Tuberculosis," Nos. (16 and 19) 62, 69 (97, 99 and 100), 122, 274, 276, 291, 377, 382, 389 for other cultures from abdominal organs.

TUBERCULOSIS OF BONES AND JOINTS.

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
202	Pus	H.Type 4	Non-virulent	M.S.	?	?	?	Tuberculosis of Hip
203	"	H.Type 1	"	E.S.	?	?	?	"

(202) History. Not obtainable.

(203) History. Not obtainable.

GENITO-URINARY TUBERCULOSIS.

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
199	Kidney	B.Type 1	Virulent	L.C.	26 yrs.	F.	3 mos.	Tuberculous Kidney
215	Urine	H.Type 1	Non-virulent	?	?	?	?	?
372	"	"	"	Miss S.	21 yrs.	F.	6 mos.	Tuberculous Kidney

(199) History. Began April 4, 1908, suddenly with frequent micturition, pain in the bladder and attacks of pain in the region of the right kidney. No tuberculosis elsewhere. Catheterization of the left ureter resulted in the withdrawal of normal urine, while that from the right ureter contained pus cells, red blood cells, casts and tubercle bacilli. The right kidney was removed July 7, 1908. About one-fifth of the kidney substance had been converted into an abscess which opened into the pelvis of the kidney. Scattered through the rest of the organ, but more especially in the region of the abscess, were small tubercles. Left the hospital and has been apparently perfectly well since the operation.

(215) History. Not obtainable.

(372). History. Has always been strong and well. There is no tuberculosis in her family. About six months ago there appeared an acute cystitis which lasted for 10 days, and during which there was some slight hæmaturia. About two months later the symptoms recurred. This time they were much worse and lasted six weeks. The patient seems to be in good condition. An acid fast bacillus was found in the urine.

SUMMARY OF RESULTS

Diagnosis of Cases Examined*	Adults (16 yrs. or over)		Children (5 to 16 yrs)		Children (under 5 yrs.)	
	Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Pulmonary Tuberculosis...	225	—	8	—	2	—
Tuberculous Adenitis (Cervical)...............	2	—	14	4	4	6
Tuberculous Adenitis (Inguinal)	—	—	2	—	—	—
Generalized Tuberculosis..	2	—	1	—	16	4
Tubercular Meningitis.....	—	—	1	—	4	—
Abdominal Tuberculosis...	—	—	1	1	—	1
Tuberculosis of Bones and Joints.............	—	—	2	—	—	—
Genitourinary Tuberculosis	1	1	1	—	—	—

For ease of reference to the bovine viruses the following list is appended. They are classed as bovine by reason of their cultural characteristics and virulence for rabbits.

Tuberculous Adenitis (children), 96, 163, 184, 216, 217, 247, 260, 262, 381, 393.
Generalized Tuberculosis (children), 62 (63 and 69), 66 (122 and 123).
Abdominal Tuberculosis (children), 109, 433.
Genito-urinary Tuberculosis, 199.

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*In a few instances where the age is not accurately known, the approximate age has been used in this tabulation, though the age is given as (?) in the other tables.

VIRUSES FROM MILK

No.	Material.	Cultures.	Rabbit Virulence.	Remarks.
8	Milk	B. Type 1	Virulent	
12	“	“	“	
14	“	“	“	
89	“	“	“	
111	“	H. Type 1	Non-virulent	Human Type, apparently market contamination

VIRUSES—BOVINE ORIGIN (Controls from cattle)

259	Lung	B. Type 1	Virulent	
271	Lymph Node	“	“	
310	Ing. Node	“	“	
311	Mes. Node	“	“	
312	Bron. Node	“	“	
313	“	“	“	
314	“	B. Type 3	“	
315	“	B. Type 1	“	
317	“	“	“	
321	Lung	“	“	
322	Bron. Node	“	“	
323	“	“	“	
324	Lung	“	“	
327	Bron. Node	B. Type 3	“	
329	Tissue	B. Type 1	“	
330	“	“	“	
331	“	“	“	
332	“	“	“	
334	“	B. Type 2	“	
489	Bron. Node	B. Type 1	“	
490	“	“	“	
491	“	“	“	
493	“	“	“	
495	Lung	“	“	

SUMMARY OF CALF INOCULATIONS

Virus.	Diagnosis.	Weight of Calf	Autopsied after.	Weight at Autopsy.	Autopsy.
No. 64 (See No. 125) Tonsils	Pulmonary Tuberculosis (Adult)	190 lbs.	142 days (killed)	267½ lbs.	Gain in weight, 77½ lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 118 Sputum	"	185½ lbs.	140 days (killed)	269 lbs.	Gain in weight, 83½ lbs. Caseous area at site of injection. Tubercles in prescapular lymph node.
No. 15 Sputum	Pulmonary Tuberculosis (Children)	119 lbs.	142 days (killed)	174 lbs.	Gain in weight, 55 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 116 Cerv. Nodes	Tuberculous Adenitis (Children)	189½ lbs.	142 days (killed)	273 lbs.	Gain in weight, 83½ lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 150 Cerv. Nodes	"	218 lbs.	142 days (killed)	284 lbs.	Gain in weight, 66 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 155 Cerv. Nodes	"	263 lbs.	142 days (killed)	372 lbs.	Gain in weight, 109 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 163 Cerv. Nodes	"	156 lbs.	73 days (died)	126 lbs.	Loss in weight, 30 lbs. Progressive generalized tuberculosis.
No. 217 Cerv. Nodes	"	203½ lbs.	23 days (died)	134 lbs.	Loss in weight, 69½ lbs. Progressive generalized tuberculosis.
No. 262 Cerv. Nodes	"	158½ lbs.	53 days (died)	136 lbs.	Loss in weight, 22½ lbs. Progressive generalized tuberculosis.
No. 19 Spleen	Generalized Tuberculosis (Children)	262½ lbs.	140 days (killed)	358 lbs.	Gain in weight, 95½ lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 20 (See 16) Bron. Nodes		133 lbs.	140 days (killed)	195 lbs.	Gain in weight, 62 lbs. Pocket of fibrous tissue at site of injection, surrounded by caseous cervical nodes.

SUMMARY OF CALF INOCULATIONS

Virus.	Diagnosis.	Weight of Calf.	Autopsied after.	Weight at Autopsy	Autopsy.
No. 58B (See 58A) Cer. Sp. Fl.	Generalized Tuberculosis (Children)	129½ lbs.	140 days (killed)	204 lbs.	Gain in weight, 74½ lbs. Caseous area at site of injection. No other tuberculous lesions
No. 62 Mes. Nodes	"	125 lbs.	125 days (killed)	206 lbs.	Gain in weight, 81 lbs. Regressive generalized tuberculosis. Generalization marked.
No. 74 Cer. Sp. Fl.	"	157 lbs.	140 days (killed)	204 lbs.	Gain in weight, 47 lbs. Caseous area at site of injection. Mass of caseous nodes in ligament at junction of two gastric pouches.
No. 98 (See No. 97) Lung	"	204 lbs.	142 days (killed)	288 lbs.	Gain in weight, 84 lbs. Two caseous areas at site of injection. No other tuberculous lesions.
No. 99 Spleen		242½ lbs.	142 days (killed)	313 lbs.	Gain in weight, 70½ lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 100 Mes. Nodes		226 lbs.	142 days (killed)	332 lbs.	Gain in weight, 116 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 148 Cer. Sp. Fl.	"	190 lbs.	142 days (killed)	246 lbs.	Gain in weight, 56 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 2 (See No. 4) Sputum	Tubercular Meningitis	197½ lbs.	142 days (killed)	267 lbs.	Gain in weight, 69½ lbs. Caseous area at site of injection. No other tuberculous lesions.
(No. 61 Cer. Sp. Fl.	"	109 lbs.	140 days (killed)	142 lbs.	Gain in weight, 133 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 109 Mes. Nodes	Tubercular Peritonitis (Child)	152 lbs.	24 days (died)	120½ lbs.	Loss in weight, 31½ lbs. Progressive generalized tuberculosis.
No. 310	Bovine Controls	218 lbs.	20 days (died)	163 lbs.	Loss in weight, 55 lbs. Progressive generalized tuberculosis.
No. 491	"	121 lbs.	40 days (died)	110 lbs.	Loss in weight, 11 lbs. Progressive generalized tuberculosis.

THE INCIDENCE OF TUBERCLE BACILLI IN NEW YORK CITY MILK

With a Study of Its Effects on a Series of Children

Alfred F. Hess, M.D.

One of the most important questions to-day which confront those fighting the spread of tuberculosis is: What proportion of infection is human, what proportion bovine in origin? It is not necessary for me to review in this place the various answers that are given this question by sincere and capable workers in the same or different countries. In the United States we find Theobald Smith[1] stating that, in his opinion, not over 1 per cent. of human tuberculosis is of bovine origin, and, on the contrary, others—for example, Ravenel[2]—believing that a considerable percentage of the disease is of this origin. The British Tuberculosis Commission recently reported that "a very considerable amount of disease and loss of life, especially among the young, must be attributed to the consumption of cow's milk containing tubercle bacilli." The opposite view of Professor Koch is known to all. In view of such conflicting statements on a subject of such vital importance, physicians and laymen are, at the present time, undecided whether they should concentrate their energies against the dangers from the tuberculous individual, or whether they must guard against a dual source of infection. Prophylaxis suffers from this indecision.

Scope of the Investigation

My work was undertaken with a threefold object: 1, probing one of the elemental phases of this broad question, to ascertain how often milk supplied to a large city, such as New York, contains virulent tubercle bacilli; 2, to study the nature of these bacilli, to learn whether they conformed uniformly to the bovine type as was to be expected considering their source; and, finally, 3, to report on the health of some children who consumed milk containing virulent bacilli.

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[1] Smith, Theobald: The Channels of Infection of Tuberculosis, Boston Med. and Surg. Jour., Sept. 26, 1907.

[2] Ravenel: Etiology of Tuberculosis, Am. Jour. Med. Sc., October, 1907.

I realized that the presence of tubercle bacilli in milk did not necessarily furnish an index of the frequency of infection of those who drink such milk. Nevertheless, it shows the frequency of exposure to this infection, so that it is important that we should at the outset have an approximate idea of the incidence of such bacilli in the market milk. I found experiments of this nature few in number and conflicting in results. The milk of New York City had never been investigated in this particular, and examinations which had been made elsewhere were, in the main, incomplete or dated back to a period when our milk was obtained under quite different conditions from those which exist at the present time. However, since I began this investigation more than a year ago, a comprehensive report of the tuberculous contamination of the milk supply of the city of Washington has appeared,[3] to which I shall refer in detail. The literature of this subject will be found well abstracted in this publication.

As I have mentioned, the second part of my investigation consisted in isolating in pure culture some of the tubercle bacilli found in the milk, and in differentiating them according to type. This was undertaken in order to learn whether all the cultures would prove to be bovine, or whether possibly we might find human or intermediate varieties. It seemed also desirable that more strains should be isolated from bovine material, in order to judge more certainly of the distinction between the two types of bacilli.

The material was obtained in the following manner: In the tests of raw milk, specimens were gathered only from the large 40-quart cans and not from bottled milk. This was done, partly because the facilities of the health department rendered it simpler, but more especially because more than one-half of the milk sold in New York is not bottled, but constitutes the so-called "loose milk." The fact that unbottled milk forms such a large proportion of the municipal supply is sufficient in itself to warrant its selection as a basis for examination. Furthermore, loose milk and bottled milk are frequently identical, are dispensed by the same dealer and derived from the same dairy. The specimens were collected in the same manner as those brought to the laboratory twice a week for general bacteriologic examination. I used the same sterilized 1-ounce bottles with a tight-fitting tin cap. The milk was taken indiscriminately from grocery stores, dairies and large plants in various quarters of the city. Two specimens were dipped out from each can, one for the bacterial count, another for examination for tubercle bacilli. Six to twelve were collected at a time for my purposes, although one hundred were obtained by the inspectors for general examination. In most cases I obtained the milk myself, and my selection was guided merely by an attempt to obtain specimens from dealers who had small children who drank this milk in a raw state. This was done with the object of determining the subsequent health of these children, which study forms the basis of the third part of this paper.

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[3] Anderson: U. S. Hyg. Lab. Bull. 41.

Investigation of Tubercle Bacilli in Milk

In the laboratory the specimens were kept in the refrigerator. Before being opened they were thoroughly shaken and then 10 c.c. of milk were removed by means of a sterile pipette and transferred to a small tube, in which it was centrifuged from five to seven minutes. Two smears were made from the surface of the cream which formed a solid layer at the top of the tube. The entire cream layer, which varied in amount, was removed by means of a sterile copper spoon and placed in a Petri dish. The skimmed milk was poured away and disregarded with the exception of the lowest cubic centimeter, which was poured into another dish. Two smears were made from the dregs in the centrifuge tube.

These two portions of milk, the cream and the lowest cubic centimeter, were drawn into small antitoxin syringes and injected into two guinea-pigs. It was necessary to dilute the cream with a small quantity of salt solution in order to render it fluid. As far as possible unused pigs of medium weight were employed; in many instances, however, I had to use larger pigs which had served as tests for diphtheria antitoxin. In order to make certain that such pigs were not previously tuberculous, autopsies were performed on fifty which had been used in this work. In no instance was any tuberculous infection encountered. In the early tests the inoculations were made into the muscles of the thigh, following the recommendation of Ostertag.[4] Later, however, subcutaneous inoculations into the groin were resorted to, as the disadvantages of the intramuscular method outweighed its advantages. It was found that the deep injections at times gave rise to hematomata at the site of inoculation, that they were sometimes followed by sudden death, and that the inguinal glands enlarge less markedly than when the pigs were inoculated subcutaneously. For each inoculation a different syringe and needle were used. The pigs inoculated on the same day were kept in the same cage. Very few abscesses developed, probably owing to the fact that almost three-quarters of the inoculations were made deep into the muscles; those which did form did not ulcerate through the skin. The animals were examined twice a week. They were not killed after a definite period, but in some instances, in which cultures were not desired, they were allowed to live long after they were known to be tuberculous. In this way we could judge somewhat of the dosage and the virulence of the tubercle bacilli in the milk.

One hundred and twelve specimens of raw milk were tested in the manner described, but as in five instances the animals died within two weeks of inoculation or were lost in other ways, only 107 came under consideration. Table 1 gives a summary of the results. Nineteen specimens were found to contain tubercle bacilli. It was found, however, on tracing all these samples to their source, that two were duplicates, so that we must consider that the positive inoculations numbered only seventeen. Thus the figures resolve into seventeen positive tests out of 107, which means that 16 per cent. of the milk contained tubercle bacilli.

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[4] Ostertag: Untersuchungen über die klinische und bakteriologische Feststellung der Tuberkulose des Rindes, 1905.

TABLE 1.—DATA OF TUBERCULOSIS MILK SPECIMENS.

Date.	Tuberculous Specimens.	Tuberculous Sediment or Cream.	Bacteria per c.c.	Designation of Wholesaler.	Wholesalers Who Sell Similar Milk in Bottles.
10/24	1	*Sediment	390,000	A	0
11/7	1	“	4,800,000	B	1
	1	Cream and Sed.	80,000	C	1
11/12	1	Cream	170,000	W	1
	1	Cream and Sed.	90,000	W	1
11/19	1	“ “ “	80,000	E	1
	1	“ “ “	00,000,000	B	1
11/25					
12/5	1	Sediment	50,000	O	0
	1	Cream	1,400,000	G	1
	1	“ and Sed.	20,000	B	1
	1	“	60,000	B	1
	1	*Sediment	00,000,000	B	1
12/19					
12/26					
12/31	1	*Sediment	00,000,000	E	1
	1	Cream and Sed.	8,000,000	H	0
	1	*Cream	230,000	B	1
1/6	1	“ and Sed.	160,000	J	1
	1	“ “ “	180,000	J	1
	1	*Cream	60,000	J	1
	1	“ and Sed.	00,000,000	J	1
	19				
Excluding duplicates	17				

In every one of these seventeen cases, as well as in the two duplicates, tubercle bacilli were found in the guinea-pig at autopsy. The case was looked on as negative unless the bacilli were shown to be present. For example, the autopsy of pig 436, killed three months after inoculation, reads as follows:

At the time of autopsy these lesions were considered definitely to indicate tuberculosis, but as bacilli could not be found, and reinoculation tests proved inconclusive, the case was rejected. In most instances reinoculation into a second series of pigs was made (Table 2). In order to control the results still further and avoid the possibility of confusing the tubercle bacillus with similar acid-fast organisms, cultures were made in each instance. In those cases in which serum or

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*Animal inoculated with other part of milk lost or died.

egg media was not employed—in other words, in which the object was not to cultivate the tubercle bacillus—glycerin agar was used. In no instance was an acid-fast organism encountered.

TABLE 2.—DATA OF MILK INOCULATIONS

Date.	No. Samples.	No. Pigs Inoculated.	No. Dying within 14 Days.	Otherwise Lost.	Secondary Inoculations.
10/24	12	24	2	2	2
11/7	12	24			6
11/12	12	24			2
11/19	12	24			4
11/25	6	12			4
12/5	12	24	1		6
12/19	6	12	1		
12/26	6	12	1	1	
12/31	12	24	1	3	
1/6	10	20	4	3	2
2/5	12	24		1	
Total	112	224	10	10	26
Net total	107				

(In five instances both pigs were lost or died.)

Somewhat emaciated, large bilateral inguinal glands; mesenteric glands hard, size of small marble; slight enlargement of iliac and retroperitoneal glands; spleen three times normal, containing a caseating abscess and miliary tubercles. Liver showed miliary tubercles, lung negative.

The smears made from the cream and from the sediment, of which mention has been made before, showed no tubercle bacilli. In no slide could they be definitely made out, although in those instances in which animal inoculations demonstrated tuberculosis the slides were carefully examined for a second time. They showed a large number of bacteria and leucocytes, roughly about an equal number in cream and sediment, but the number was found to bear no relation to the presence or absence of tubercle bacilli in the milk (Table 1).

I next examined some "commercially pasteurized" bottled milk, making use of the same technic. Eight samples were obtained from three different plants, with the intention of using more samples should these first tests give a negative result. The dealers in this milk state that it has been subjected to a temperature of 160° F. for forty seconds. In one of the eight samples tubercle bacilli were demonstrated by animal inoculation, which proves that this method, in practice, furnishes inadequate protection. Investigation on this particular was pursued no further.

Human and Bovine Tubercle Bacilli in Milk

Before discussing the results which we have outlined above, it may be well to give a brief account of the cultures of tubercle bacilli which were isolated. The details of this laboratory study will be published elsewhere. The method used was that employed by me in former work in this field,[5] [6] For the first generation dog serum or egg medium was used. The morphology of the bacilli, facility for cultivation, and virulence toward rabbits were considered in the differentiation of the human from the bovine type. The ability of a culture to induce a fatal generalized tuberculosis in the rabbit was looked on as evidence of its being bovine in nature. Eight cultures were isolated in all; in two other cases cultures were attempted but failed. From a practical standpoint it is of importance that, although seven proved bovine in type, in one case a human strain was isolated. As far as I am aware this is the first instance in which contamination of milk with the human type of tubercle bacillus has been demonstrated. The actuality of this source of infection, therefore, can no longer be denied; it must be considered in the safeguarding of our milk supply. On the farm, at the dairy, in the retail store, in the varied path from cow to consumer, the tuberculous individual must not have access to the milk.

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[5] Hess: An Examination of Excised Tonsils. Arch. Pediat., January,
[6] Hess: Primary Tuberculosis of the Mesenteric Glands, Am. Jour. Med.
1908, p. 31.

TABLE 3

DATA OF INTRAVENOUS INOCULATION OF RABBITS WITH PURE CULTURES OF TUBERCLE BACILLI

	Total Age of Culture.	Generation.	Age of Culture.	Amount Inoculated.	No. Rabbit.	Result	General Remarks.
Culture I......	82 days	3d	22 days	0.5 c.c.	320	Chloroformed 21 days (ill)	General Tuberculosis
" II.....	95 "	4th	20 "	0.5 "	321	Died 18 days	"
" III....	76 "	3d	25 "	0.5 "	325	" 16 "	"
" IV.....	67 "	3d	28 "	0.4 "	326	" 20 "	"
" V......	98 "	4th	21 "	0.5 "	327	" 23 "	"
" VI.....	79 "	3d	30 "	0.5 "	331	Chloroformed 17 days (ill)	" "
" VII....	69 "	3d	26 "	0.5 "	332	Died 15 days	"
" VIII	21 "	3d	63 "	1 mg.	181	Killed 80 days	Few tubercles in lungs and kidneys
	21 "	2d	42 "	1 "	521	Alive 63 days	Gained in weight

Characteristics of the Eight Cultures Isolated

The cultures I-VII must be classed as bovine in type, not only on account of the marked virulence to rabbits which they exhibited, but also because of their cultural characteristics. They grew very sparsely, showing for some generations only a fine veil-like growth, which it was very difficult or impossible to transfer successfully to glycerin broth. They were markedly similar in all these properties, only one standing apart from the others in that it showed a more abundant growth in the first generation. However, even this strain did not grow profusely when compared with cultures of the human variety.

Culture VIII is of especial interest and seems worthy of detailed description. The original material was obtained from a can of milk in a small grocery store. It was inoculated subcutaneously into two guinea-pigs, both of which developed generalized tuberculosis. From one of these animals tissue was inoculated into another pig (No. 855), from which cultures were made upon egg media about two months later. In order not to lose the material, tissue was once more transferred into a pig (No. 972), and further cultures carried out after the same interval. These two sets of cultures proved to be identical; after three weeks a vigorous confluent growth was obtained on glycerin egg. These results were so unexpected that a rabbit (No. 625) was inoculated with one of these strains and cultures were made from its tissues. By using the rabbit instead of the highly susceptible guinea-pig we made certain of selecting for culture the most virulent bacilli. These strains also grew vigorously in a manner characteristic of bacilli of the human type. Reference to the virulence tests of the table shows that this strain (Culture VIII), in strong contrast to all the others, possessed but feeble virulence for rabbits. One of the rabbits when inoculated weighed 1.740 gm., and when killed had gained 550 gm.; the other weighed 1.710 gm., and now after a period of 63 days, weighs 1.820 gm. Thus this culture, in contradistinction to the others, must be regarded as belonging to the human type, both on account of its facility for cultivation and its comparatively feeble virulence.

From a study of these eight cultures we must emphasize the sharp contrast which usually exists between tubercle bacilli of the human and those of the bovine type, isolated from cattle, a distinction which in the instances here reported was absolutely diagnostic and incapable of misinterpretation. Furthermore the results are instructive from another point of view, for, for the first time, they bring forth an instance where tubercle bacilli of the human variety have been isolated from milk and thus point out another source of danger from contamination by the tuberculous individual.

The percentage of milk found to contain tubercle bacilli, namely, 16 per cent., is so high that we feel that it requires further comment. It must be admitted that the milk chosen for examination, commonly known as "loose milk," is somewhat below average grade. In this fact, however, we can find little comfort, for subsequent inquiry revealed that of the nineteen tuberculous specimens fifteen were sold by dealers who furnish bottled milk coming from the same farms (Table 1). This shows that an examination of the average bottled milk would reveal very nearly the same figures. Another matter of practical interest concerns the diffusion of the bacilli throughout the milk. If several samples were taken from the same can, would all or only some show the presence of tubercle bacilli? This question, which was constantly in mind, was answered by a chance control experiment. In some instances the samples were taken at random from those sent to the laboratory by the inspectors. It so happened that of the 10 samples submitted on January 6, four proved to be contaminated with tubercle bacilli (Table 2). Recently, on inquiring into the source of these samples, it was learned that the four were taken from two cans in the same plant—in other words, that two were duplicates. This method of control specimens is resorted to by the Board of Health to compare the results of the bacterial counts. In this instance it served as an unconscious control of my work. Of the eight guinea-pigs inoculated with these four specimens, seven developed tuberculosis, one dying prematurely of a secondary infection. As only 10 c.c. of milk were used for the test in each instance, that is, less than one tablespoonful, this would lead us to conclude that these two 40-quart cans were thoroughly permeated with tubercle bacilli, and that there was little possibility of any one partaking of the milk without at the same time partaking of virulent bacilli. Some other of our results lead to the same conclusion: The fact that on November 12 (Table 1) two positive specimens were obtained which emanated from the same wholesaler, although retailed by different stores on the same block, and that on December 5 this was the case in three instances, demonstrates that a considerable portion of the milk sold by the wholesalers on those dates contained tubercle bacilli.

Without attempting a review of the literature we should like to call attention in this connection to the results of some others in similar investigations. In the comprehensive test of the milk supply of the city of Washington, reported this year by Anderson, 6.72 per cent. of the samples were found to contain tubercle bacilli, a percentage considerably lower than that which I report for New York. This wide variation can be accounted for in part by differences in the technic which was employed. In the Washington tests usually only one animal was used for each sample, and, second, the cream was not injected. Had these methods been followed, our percentage would have been considerably lower. This may be judged by the fact that of the 19 tuberculous specimens only nine produced tuberculosis in both of the injected animals (Table 1). In five instances this was due to the premature death of one of the pigs. It may be said in general that the more animals used for testing each sample the greater the chance of finding tubercle bacilli. The other point of difference in technic is more important. As bacteria collect in the cream as well as in the sediment, it is evident that unless we test both portions some may be overlooked. It will be noticed that in two of our cases bacilli were demonstrated in the sediment alone, in three others in the cream alone (Table 1). The animals injected with the negative portion of these samples developed either no lesions or such as were inconclusive. Had we not injected the cream, three of the positive cases would have been reported as negative, which in itself would have reduced our figures from 16 per cent. to 13 per cent. Moreover, higher percentages than we have obtained have been published by others. For instance, Rabinowitch and Kempner[7] found, in an examination of 25 samples of Berlin milk, that seven samples, or 28 per cent., contained tubercle bacilli. And again, in an examination by Macfadyen[8] at the Jenner Institute, 17 among 77 specimens, or 22 per cent., were found to be infected with virulent tubercle bacilli.

Condition of Children Who Drank Tuberculous Milk

So much concerning the laboratory part of this study. We have shown how frequently tubercle bacilli are present in the milk of a great city; how, although generally bovine in type, they may be of the human variety. Let us now consider the condition of some children who drink this milk. As I mentioned above, samples were gathered in most instances from such dealers as had children. Each store-keeper was asked whether there were children in the family, and, if so, whether they were given raw milk from the can in the store. Of the 17 dealers from whom milk was obtained which contained tubercle bacilli, ten belonged to this category. These ten had 18 children. As Table 3 shows, nine of these were two years of age or under, and only one over five years of age. The health of these children has been followed for the past year, that is, since they are known to have ingested virulent tubercle bacilli. They have been visited by me from time to time, and finally during the past fortnight 16 were tested with tuberculin and subjected to a more or less complete physical examination. The conjunctival tuberculin test was employed, as it was feared that the cutaneous test might not be permitted. The parents were told the reason for this medical supervision, and in only a few cases offered objections. The results, which are given in tabular form (Table 3), were briefly as follows:

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[7] Rabinowitch and Kempner: Ztsch. f. Hyg., 1899, xxxi.

[8] Macfadyen: Lancet, London, 1899, ii, 849.

TALE 4.—DATA CONCERNING CHILDREN WHO DRANK MILK CONTAINING TUBERCLE BACILLI

Milk Dealer.	No. of Children.	Age Years.	Conjunctival Test.	Condition One Year Later.
A	2	1	—	Healthy
		3	—	"
B	1	1½	—	"
C	2	2	—	Poorly nourished
		4	—	Healthy
W	3	5/12	o	"
		2½		
		5	+	Poorly nourished. Physical exam. and family history doubtful.
E	1	½	—	
B	1	2	+	Poorly nourished. Cervical adenitis incised 4 mos. ago. Von Pirquet positive. Family history negative.
G	3	3	—	Healthy
		5	—	"
		9	+	" Family history negative.
B	1	5/12	o	Healthy
B	2	1½	—	Poorly nourished
		3½	+	Healthy. Physical exam. and family history negative.
O	2	2	—	Healthy
		4	—	"
	18		4	

Of the 16 children tested with tuberculin, four reacted; two of these were poorly nourished, but showed no definite signs of tuberculosis; the other two were healthy. One of the cases was of especial interest:

+ = positive reaction.
— = no reaction.
o = no test.

This child consumed daily a pint of the poorest milk examined (B. Table 1). She was two years of age, poorly nourished, and five months ago developed a cervical adenitis, which was incised at a dispensary. She reacted to both the conjunctival and von Pirquet tests. No other member of the family appeared to be tuberculous.

We realize that we can not draw sweeping deductions from an experiment of this nature. It lacks the precision of laboratory work, a weakness inherent to a test on the human subject. It may well be said that we know neither how often the children drank milk containing tubercle bacilli nor the number of bacilli consumed. In answer to this we can merely state that 10 c.c. of this milk showed the presence of virulent bacilli; that these bacilli were found to be thoroughly distributed throughout the milk, and that the children in most instances consumed a pint or even a quart of such milk daily. Moreover, as this is the only investigation of the kind which has been made, and which it seems possible to pursue, it seems worthy of consideration.

First and foremost it is evident that as almost all the children who drank the milk were later found to be in good health, we may conclude that milk containing tubercle bacilli does not necessarily, nor even usually, excite tuberculosis. The striking frequency with which we found bacilli would of itself force this conclusion on us. For with 16 per cent. of the "loose milk" infected, if infection were coincident with exposure, it would mean that on an average every child who drank this raw milk daily for a week would show some tuberculous lesion. Tuberculin tests and postmortem examinations on infants prove the fallacy of such reasoning. On the other hand, the occurrence of a case of cervical adenitis, one of the most frequent forms of bovine tuberculosis, in an infant known to have drunk milk containing bovine tubercle bacilli, who reacted to tuberculin although surrounded by no source of tuberculous infection, is certainly suggestive of bovine infection. We hope to follow the course of this child and some of the others so as to judge of their future welfare.

It seems to us, judging from our own investigations and those of others, that, at the present time, the relation of bovine tuberculosis to man may fairly be stated as follows: 1. This type of bacillus, although less virulent than the human variety, is capable of infecting human beings. 2. Children are more susceptible than adults to bovine infection. Numerous instances of tuberculosis of the tonsil, of the cervical and of the mesenteric glands have been proved to have been incited by the bovine bacillus. Among others, Theobald Smith, Fibiger and Jensen, the German and the British Commissions, and myself,[5] have reported cases of this nature. Recently I was able to collect reports of 44 cases of undoubted bovine infection of the mesenteric glands, 41 of which occurred in children.[6] In this connection a case taken from this year's Medical Report of the County of Aberdeen, and sent to me by Dr. Adams, seems worthy of summary. A farm servant who had three daughters, aged 9, 6 and 4 years, lost the eldest on January 7, 1907, with symptoms of meningitis. In the beginning of March the youngest became ill and died on the 18th with similar symptoms. An autopsy showed tuberculous meningitis and tuberculosis of the mesenteric nodes. In the cerebrospinal fluid tubercle bacilli were found. There was no history of tuberculosis on the father's or mother's side. The milk which these children drank came from one cow, which was found to have tuberculosis of the udder, and was killed and shown to be suffering from extensive generalized tuberculosis. Careful cultural examination of bacilli from the cerebrospinal fluid of the child, as well as from the milk and from the mesenteric glands of the cow, were made by Dr. J. Milner Adams, of the University Pathological Department, and all found to be bovine in type and similar in their microscopic, cultural and pathogenic characteristics. Neither this well-studied case nor the figures given above are, indeed, sufficient to prove that bovine tuberculosis is the "great white plague" which we are all fighting, but they do show, without doubt, that this bacillus is fraught with danger to children. Even the stanchest supporter of Koch would not care to have his children drink milk from a cow which he knew to be giving forth tubercle bacilli.

As yet there is no sufficient basis for a statistical estimate of the importance of bovine tuberculosis, but we must remember that even if, with Theobald Smith, we charge it with but 1 per cent. of the total tuberculous infections, this small percentage is sufficient to establish it as a disease worthy of active recognition. To appreciate the force of the plea for recognition of such a small percentage we have but to reflect that tuberculosis claims in the United States every year approximately 200,000 persons and that the total number of infections amount to twice this number. Thus, this small percentage is sufficient to cause the yearly number of cases of bovine infection of human beings in this country to extend into the thousands. It is solely because this type of tuberculosis is submerged by the 90-odd per cent. of human tuberculosis that it is now threatened with losing its identity. If it were possible to diagnose bovine infections by clinical methods, and if the total number were grouped under a different name, instead of being included in the generic term "tuberculosis," we would have a disease of sufficient magnitude to preclude all questions as to the advisability of active preventative measures. Were there as great a number of human beings infected, for instance, by the foot-and-mouth disease of cattle, the matter would be considered as requiring state or federal attention. So that, to summarize, without ever losing sight of the fact that the bacillus of the human type is the cause of the great scourge, the scourge of tuberculosis, it would seem wise, also, to regard the bovine bacillus as the cause of an unquestionable, although comparatively small number of tuberculous infections.

Finally, a word as to the practical lesson which this investigation teaches. It is not new; it has been emphasized with ever-increasing earnestness by many students of the problem. It is to be hoped, however, that the alarming degree of tuberculous contamination found in the New York City milk, and the concrete figures which I have furnished, may prove an effective argument for those who are striving with the legislators in the cause of pure milk. It may be thought that, as I used mixed milk in the tests, it is possible that the bacilli were, in many instances, given off by one infected cow in a herd and were diffused throughout the supply. We can not well fall back on this comforting explanation, when we reflect that a recent report by Moore[9] showed that of 421 herds in New York State tested with tuberculin, 302 contained reacting animals. This high percentage of disease harmonizes with the high percentage of contamination which I found in the city milk. Again, the investigations of Schroeder[10] showing how frequently feces are present in milk, and how frequently tubercle bacilli are present in the feces of apparently healthy cattle, point clearly to a widespread dairy infection. In view of these facts, it would seem wise as an immediate safeguard, thoroughly to pasteurize or to boil all milk not coming from tuberculin-tested cows. "Commercial pasteurization" does not protect against tubercle bacilli; it should not bear the name of pasteurization, or, at most, this milk might be termed "partially pasteurized milk."

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[9] Moore: Can Tuberculosis be Eliminated from Cattle? New York State Jour. Med., 1908, viii, No. 5.

At the same time that we are carrying out these temporary expedients, those who are urging broader remedial measures, such as adequate inspection and testing of the dairy herds, should be accorded the support of phsicians and laymen alike. For if New York City had been granted, instead of 47 inspectors of farms, 150 such inspectors, it is certain that 16 per cent. of the milk sold in its precincts could not be found to harbor virulent tubercle bacilli.

Summary

Virulent tubercle bacilli were found in 17 among 107 specimens, that is, in 16 per cent. of the milk retailed from cans in New York City. These bacilli were not remonstrable by direct microscopic examination, but were proved to be present by means of animal inoculation. Cream as well as sediment was found to harbor these organisms, so that in all experiments of this nature these two parts of the milk should be used in making inoculations.

Tubercle bacilli were likewise found in a specimen of "commercially pasteurized" milk, showing that this method, as now carried out, does not insure protection in this particular. It is suggested that, as as this name is misleading, only such milk be labeled "pasteurized" as has been heated for a length of time and to a degree of temperature sufficient to render it an absolutely safe food.

When the tubercle bacilli were isolated they were found to be in all but one instance bovine in type. In this instance, however, a human variety was differentiated, which shows that milk may become infected from tuberculous individuals and that this source of contamination should be guarded against.

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[10] Schroeder: The Unsuspected but Dangerously Tuberculous Cow, Bureau of Animal Industry, Cir. 118.

A number of infants and young children who drank milk containing tubercle bacilli, when examined one year later, seemed to be in average health. A fourth of the number, however, reacted to tuberculin. One of these subjects was in poor physical condition and had suffered from a recent glandular infection.

Although probably over 90 per cent. of tuberculosis is due to infection from human beings, it is believed that we are not, therefore, justified in neglecting the danger from bovine infection, for even a small percentage of the infections in the United States means thousands of cases of tuberculosis.

As an immediate safeguard, milk not coming from tuberculin-tested cows should be pasteurized or brought to a boil. Many additional inspectors should be allowed for examining the herds, and, finally, all cows should be tested with tuberculin, and animals which react should be condemned or isolated.

ON THE TYPE OF CULTURES FROM OLD CUTANEOUS TUBERCLES OF BUTCHERS

By Alfred F. Hess, M.D.

The studies thus far carried out in different countries, using the same methods of differentiation, agree in charging the human type of tubercle bacillus with an overwhelming preponderance of human infections. In this connection, however, there is one disturbing criticism that is now and again brought forth to shake the confidence imposed in these conclusions, based as they are on the differentiation of the types of bacilli. It is claimed by some capable workers that it is possible for the bovine bacillus to be so altered by years of sojourn in the human tissues that its essential characteristics become altered and it simulates the human type of bacillus. Without doubt if this metamorphosis can readily take place some of those infections which we now believe to have been of human origin may well have had their source in bovine infections of years ago, and it is to no purpose to designate them as human. In fact, if this be so, the entire method of differentiation which has accomplished so much in the past decade and promises so much more in the practical and theoretical study of tuberculosis, would have to be abandoned as not only unreliable but misleading.

The main difficulty in studying this question in man is that we are rarely certain of the nature of the original infection and accordingly cannot judge of the change of type. Our main reliance is generally circumstantial evidence. An exception to this rule, however, is furnished by those few cases of inoculation tuberculosis where the bovine source of infection is definitely known. I have with difficulty found two cases of this nature, both in slaughter-house workers, and report a study of them coupled with some other experiments relating to the stability of the types of tubercle bacilli.

Case 1—This man had been employed in his present trade for seven years. Four years ago, while slaughtering an animal, he cut the middle finger of his right hand on the pointed end of a rib. He gave no thought to this injury until the found that it was slow to heal; he then merely applied various ointments. Gradually a nodule appeared at the site of the wound which assumed its present condition. He was aware of the tuberculous nature of this nodule, and in fact directed me to another worker who had the same skin lesion. I shall not enter into a minute description of the appearance of this nodule. It was the size of a large pea, brownish red and very firm in consistency. The glands at the elbow and axilla were not enlarged. The general health of the man was excellent.

Case 2—This case was very similar to the previous one. This man while slaughtering a tuberculous cow cut himself with a knife across the knuckles. This accident happened about six years ago. He remembered the date, as he was forced by this disability to quit work for some weeks and to enter the employment of another firm. As in the previous instance, a nodule appeared at the site of injury. The nodule was situated over the fourth left metacarpal joint, and resembled the tubercle described in the previous case, excepting that its surface was rougher and papilliform. This man also was healthy to all appearance; however, a large hard epitrochlear gland was palpable at the right elbow.

Both of these men were induced to have these nodules excised. After excision the surface of the growths was removed, and they were thoroughly washed in sterile water. Two guinea pigs were inoculated subcutaneously with each growth. All four animals developed tuberculosis. The cultural growth from these two cases proved to be very similar, in fact I could not distinguish between them. They both grew sparsely on egg media. They also showed a high degree of virulence for rabbits producing a generalized tuberculosis when inoculated intravenously in the dose of 1 mg. In other words, they were typically bovine.

Before discussing these results I shall describe an experiment undertaken with the object of transforming the bovine type of the tubercle ture medium resembling in its composition as closely as possible the human tissues. From the outset the inherent variance between an artificial culture medium and the living tissues was clearly realized. For this purpose, however, what may be termed a human placenta glycerin broth was devised. Human placenta was obtained under strict aseptic precautions and from this a broth was made, treating the placenta just as the beef is treated in preparing the broth for common use. One per cent. peptone, one-half per cent. salt and five per cent. glycerin were added. The entire preparation of this culture medium was conducted as sterilly as possible. In order not to destroy the complement of the human placenta, heat was applied only to 55° C. It was possible in most instances to obtain a sterile broth by means of filtration through a Berkefeld filter.

Two bovine strains of unknown virulence, one of them having been under artificial cultivation for many years, the other only recently isolated, were grown upon this culture medium for nine months, comprising eight generations. At the end of this period their virulence was found to be as marked as at the beginning, showing that in this respect they had not approached the human type. In fact, the only change that was noted was one to be anticipated from artificial cultivation of any nature, namely, a greater facility of growth in the case of the strain which had been the more recently isolated. As this experiment was very laborious and promised so little it was discontinued.

The question of the stability or variability of the types of tubercle bacilli has been discussed so frequently that I shall not review the testimony. However, in connection with this report, a few words upon this subject seem in place. From the outset it should be realized that this question has a practical as well as a theoretical aspect, and that the two need not be in absolute accord. The former considers whether it is in any way possible by natural or artificial means to convert one type of bacillus into another type; the latter whether the bacilli isolated from man and designated as human have been converted from an original bovine type. These two viewpoints must not be confused. It is true it has been demonstrated that under artificial cultivation strains may be greatly changed in their cultural and biological characteristics. Indeed, a culture isolated almost ten years ago from a cow, I recently found to simulate the human type both in luxuriance of growth and diminished virulence for rabbits. However such experiments clearly cannot be translated into practical evidence on this question. Again it may be advanced that experiments have been reported showing a conversion of human into bovine bacilli by means of passage through cattle or of bovine into avian bacilli by means of passage through birds. However, even if we accept these interpretations, and forego the criticism that has been levelled at them by others, we find these experiments to be very few in number and far outweighed by the many others where no conversion of type was effected. When we consider the practical side of this question and draw our arguments from what we know of tubercle bacilli in man, the following should be borne in mind. Atypical types have been isolated by many investigators, strains neither typically human nor bovine. This intermediate group may in part be accounted for by attributing the variation to a change of environment, for example, of bovine bacilli in the new environment of human tissue. These atypical strains, it should be remembered, are exceptional; among three bovine and four human strains which I have isolated from man, none belongs to this category. Furthermore this deviation from type cannot be regarded as evidence of the possibility of a complete change of type. In this connection it is worthy of note that typical bovine bacilli have been isolated from calcareous lymph nodes of human beings; in such instances the bovine bacilli must have existed in the human tissues for years without undergoing transformation. It has never been shown that the older the tuberculous lesion the less the likelihood of isolating bovine bacilli from man. And yet this corollary should obtain if the bacilli are readily converted in the human tissues. The question may be approached from another point of view. It is well known that no case of primary pulmonary tuberculosis has been indisputably proved to have been incited by the bovine bacillus. These cases form the great mass of tuberculosis and have been studied more than all others. If this form of the disease is due in some instances to a bovine bacillus which has been transformed in the human body, we should at least occasionally meet with this type of the bacillus in the early stage of the disease when it has not yet been converted into the human type. Not only do we know that this is not the case, but we find that the intermediate types are rarely met with in the primary pulmonary cases. So that it would seem from a practical standpoint the question of transmutation of types may well be disregarded and we can safely proceed to investigate the etiology of tuberculosis by means of differentiating the types of bacilli.

The two cases of cutaneous tuberculosis which I report claim some degree of interest because they constitute bovine infections occurring in adults. The fact that they remained localized should not be attributed to the low degree of virulence for man of the bovine bacillus, as it is well known that cutaneous affections due to the human type of bacillus, such as lupus or the "anatomical tubercle" contracted in the dissecting room, likewise do not tend to systematic invasion. Their main significance, however, lies in the fact that they furnish exceptional instances of bovine tubercle bacilli which have lived in the human tissues for years without acquiring characteristics of the human type. From this point of view they constitute evidence against the conversion in the human tissues of bovine bacilli into human tubercle bacilli. It would be interesting and of undoubted value if those who have access to similar long-standing cases of tuberculosis would make them the basis of bacteriological study.

ON THE DETERIORATION OF DIPHTHERIA ANTITOXIN

By Edwin J. Banzhaf.

Lots in duplicate of native antitoxic sera, antitoxic citrated plasma and concentrated antitoxin globulin solution were taken. One lot of each was kept at ice-box temperature, varying from 4° to 7° C. The remaining lots at room temperature (22° to 26° C.).

On starting this work it was my intention to retest these lots every two months, but, after several retests, found it was too expensive an undertaking. I therefore lengthened the retest time to six months and later to once a year. The unit value of these lots of sera was determined very carefully with a toxin that was standarized every two months against a standard test serum furnished by the Hygienic Laboratory of the Public Health and Marine Hospital Service. The deteriorations of these various lots were as follows:

For the native antitoxic sera kept in ice-box, the average deterioration for one year was 14 per cent.; for two years the average was 22 per cent.; for three years, 24 per cent.

For the antitoxic citrated plasma, kept in ice-box, the deterioration was extremely low. The average for one year was 6 per cent.; for two years, 8 per cent.; for three years, 9 per cent.

The deterioration of potency with the concentrated antitoxic globulin solution, kept in ice-box for one year was 13 per cent.; for two years, 17 per cent.; for three years, 20 per cent.

The average deterioration of potency with the native antitoxic sera kept at room temperature for one year was 18 per cent.; for two years, 24 per cent.; for three years, 26 per cent.

For the antitoxic citrated plasma kept at room temperature the average deterioration for one year was 8 per cent.; for two years, 10 per cent.; for three years, 12 per cent.

For the antitoxic globulin solution kept at room temperature the average deterioration for one year was 16 per cent.; for two years, 20 per cent.; for three years, 23 per cent.

ON SOME ERRORS IN THE DETECTION OF GONOCOCCUS IN THE VAGINITIS OF CHILDREN

IRA VAN GIESON, M.D.

When a subject has been worn as threadbare as that of the microscopic detection of the gonococcus, it seems incredible that the simple procedure of collecting the exudate in vaginitis and spreading it on a slide for microscopic examination of the gonococcus could be so faulty as often to defeat the very purpose for which it is undertaken. Nevertheless, this is a fact. It has been supposed that the transference of the exudate from the vestibule or the vagina to the slide by means of a cotton swab, such as is used in taking cultures from the throat, would be thoroughly reliable; that is, that when the secretion is spread on the slide the presence or absence of the gonococcus would indicate the presence in or the absence of the organism from the vagina.

At the Willard Parker Hospital, in the scarlet fever, diphtheria and measles wards, and in the other hospitals of the Department of Health of the City of New York, we have for years taken this procedure at its face value supposing that it gave sufficient information in determining the question of the gonococcus in its important influence in our management of this prevalent, troublesome and persistent malady in little children. Now it turns out that the method is far from providing a certain means of detecting gonococcus, and frequently fails in cases without appreciable discharge. When a method is not fully reliable it is time for the substitution of one which is more certain and trustworthy. The new pipette method proposed here is so simple that it seems like making much of a small matter in according it any extended text. But the method deals with a troublesome and a rather common malady, and as it often happens that important phenomena are disclosed and handled with methods of trivial operation, no apology is needed for a seemingly inconsequential procedure if it helps us better to detect and to manage an intractable and easily transmissible disease.

Under any circumstance specific vaginitis in children is serious

From some standpoints, it is, perhaps, less so than in adults, in that the uterus and tubes are seldom involved; in other respects, however, it is far more serious because of the danger of ophthalmia. But everyone knows that in hospitals, especially in those for contagious diseases, the disease is particularly vicious because of its liability to spread through the wards unless the greatest vigilance is exercised. We have to be on guard constantly to catch the patient at the very inception of the disease, indeed, with the detection of the gonococcus to anticipate its appearance, and equal care is imperative in not sending a child home prematurely, to infect its companions.

When vaginitis is fully developed the gonococcus determination is hardly necessary to tell us what is going on. Nor when pus is pouring out of the vagina or urethra does anyone need a microscope to know what to do or to make a diagnosis, for he would only be making sure of what he already knew. To be sure, the microscope will give the secondary information as to whether the discharge is non-specific or the contrary. In such cases, when the aid of a microscope is to a certain extent a laboratory refinement, the swab device works very well, as indeed anything else which will get the exudate spread out uniformly on the slide. The method is excellent when the disease is so obvious that its application is hardly necessary.

This whole matter of the detection of the gonococcus, regarded from a practical standpoint, becomes of value in the very beginning and the declining stages of the discharge. These are the stages where the greatest danger of widespread infection exists for the simple reason that they are not heralded and placarded with the obtrusive cardinal manifestation of the malady, namely the discharge. Here we are absolutely dependent upon the microscope, and here, where it is important to single out vaginitis cases on admission to the hospital, to apprehend the beginning cases, to detect the gonococcus when the secretion is only faintly purulent or scanty and hidden in the vaginal fornices or otherwise not obvious, here it is that the device with the swabs sometimes leaves us in the lurch.

Whether the vaginal exudations without the gonococcus, the non-specific vaginitis cases are infectious or not is exceedingly difficult to determine without inoculation experiments. From time to time we find a profuse non-gonorrhoeal vaginitis in which the leucocytes contained several small bacilli with tapering ends lying side by side. Apparently this form is infectious. Occasionally there are also non-specific cases in which there are intracellular cocci but distinctly smaller than the gonococcus. And rarely a discharge is met in which the leucocytes show no bacteria of any kind. The only organisms present are extracellular and seem to be merely the ordinary saprophytes of the healthy vagina. This form of vaginal discharge seems to be due to a simple catarrhal exudate accompanying at times measles and scarlet fever. The products of inflammation complicating the course of these exanthemata pass into the vagina and sweep out with them the ordinary vaginal bacterial flora.

The most sensible plan, I think, in the examination of these non-specific and nondescript discharges is, that while a negative report may be given as far as the gonococcus is concerned, in the question of isolating these children, we should be guided more by the general structural appearnce of the exudate than the matter of the bacterial content, because we cannot tell whether these several bacteria associated with the non-specific cases are infectious or harmless. It is difficult to describe or lay down any set rule regarding the structural appearances of the smear which indicates a dangerous or suspicious discharge, but one learns from experience that a certain preponderance of leucocytes, or an exudate almost entirely purulent justifies the diagnosis from mere vaginal hypersection (from uncleanliness, for instance), and the recommendation of isolation, even though the gonococcus is not present. A few leucocytes scattered among a large content of vaginal epithelial scales, although the smears may be laden with various kinds of bacteria, has no special significance. In the routine microscopic vaginal examinations at the Department of Health hospitals (comprising at times several hundred children) I have followed this plan of dealing with the non-specific or indifferent discharges largely according to the structural appearance of the exudate, reporting, for instance, "Gonococcus absent recommend isolation and observation for several days from largely purulent character of discharge and slight relative content of desquamated vaginal epithelium."

Considering the volume of vaginal discharge both benign and infectious, and the catarrhal conditions in measles and scarlet fever that we have to contend with, this policy has worked out very well. It places one on the safe side with many children having discharges which are not gonorrhoeal. If the discharge is benign no harm is done to the child in isolation, and, if not gonorrhoeal, but nevertheless infectious, a menace to the ward is removed. From what has been said it is apparent that adherence to a rigid rule in the absence or presence of gonococcus in the determination of this question of vaginal discharges may bring about diagnostic blunders in two ways. The observer might find organisms indistinguishable from gonococci in a vaginal secretion without the structural earmarks of a true vaginal inflammation and so condemn a child with mere profuse secretion to the vaginitis ward, and, on the other hand, he might in reporting the absence of the gonococcus leave an infectious child in the ward. Some judgment must be exercised independent of, or at least in conjunction with, the question of the morphology of bacteria in these secretions. The structural character of the secretions must be taken into consideration, and at times this is just as important a factor as the bacterial determination.

The reason for the frequent failure of the dry swab method is quite simple. Unless the discharge is profuse, the exudate on the absorbent cotton is caught in its interstices, and the result is that much of what we wish to have on the slide is retained by the cotton. Not infrequently, in thin, serous and scanty discharges, nothing but fluid is expressed from the swab to the slide, while abundant gonococci remain in the leucocytes sticking tenaciously within the meshes of the cotton fibres. In attempting to release the exudate from the preparation of the smear, the swab is rubbed and pressed about on the slide; the cellular contents may be torn off the cotton fibres with considerable mechanical injury so that the structure of the material on the slide, especially the leucocyte, is highly distorted and most unfavorable for the detection of gonococcus. Quite frequently, also, it is difficult to spread the exudate evenly and uniformly on the slides with the swab; thus the cells are heaped on top of each other and conceal the gonococci. Occasionally the narrow vaginal orifice in children wipes the surface layer of the exudate off the swab as it is being removed.

The intact leucocyte cell body is the *sine qua non* in the diagnosis of the gonococcus. Without this we lose the foothold of certainty. This is especially true of the vaginal secretion, which brings in difficulties not encountered in discharges from the male urethra. In the vaginal discharge we cannot take the free cocci into account, because, while the male urethral exudate contains as a rule no adventitious bacteria and only those of a specific or non-specific urethritis, the vaginal discharge and the healthy vagina may be contaminated with a great number of various bacteria, among them cocci, which cannot be distinguished morphologically from gonococci.

If one were to make the diagnosis on the presence of free gonococci-like organism, many children, healthy as far as vaginitis is concerned, would be wrongly put into the vaginitis ward. These smears with distorted pus cells are, therefore, misleading and often quite worthless. It is truly astonishing to discover that hundreds and hundreds of gonococci may be lost or concealed in the smears from these cotton swabs or retained on the cotton. It should not be understood that the deformity of the leucocytes is characteristic of all swab smears. It occurs extensively, however, in quite a large proportion of them when the secretion is very thin or viscous, and especially when the swab is allowed to dry in the air momentarily or through a conjunction of both factors. Occasionally the volume and consistence of the exudate is such that the film is in good condition and offers the proper conditions of accurate diagnosis.

Besides these drawbacks, it is questionable whether the swab device is wholly harmless. No one would think of using a similar procedure in the male urethra, because it would denude the ephithelial layer and damage the lining membrane. The introduction of a dry cotton swab into an inflamed and sensitive vagina, twisting it about to collect the exudate must injure the lining walls if only to a slight extent. And since, as we are told, the deeper layers of the epithelium and the membrane on which it rests act as barriers against invasion of the gonococcus into the circulation or the lymph spaces and its propagation to remoter parts, this aspect of the procedure is hardly a negligible factor. In the final and healing stages of vaginitis it is also important not to interfere with the restitution of the epithelium. If swabs must be used it would be better to wet them before they are applied; the smears are better and their introduction easier.

A very much better way to obtain the exudate is to collect it in glass tubes so that one can see what the content of the vagina is, and incidentally not lose it in transferring it to the slide. For this purpose ordinary medicine droppers are the most convenient; they are about the length of the average child's vagina and manipulation of the rubber bulb collects the secretion. Occasionally the sharp cutting edge of the dropper needs rounding off in the flame. The tubes, of course, may be sterilized and used over again, kept in bichloride solution before being applied, and the children are hardly aware of their introduction, which is more than can be said of the swab. Sometimes the pus is so scanty that instead of filling the lumen of the tube it sticks to the sides of its walls and cannot be transferred to the slide. In such instances it is well to fill the tube with a drop or two of water, or better, a 1-5000 bichloride solution before introducing into the vagina and by compressing and expanding the bulb make an emulsion of the exudate. The advantage of this is that the whole vaginal contents is obtained where the secretion is scanty, the bichloride fixes the cellular elements at the same time, and they are in perfect form when dried on the slide. Salt solution is less preferable, for it seems to contract the leucocytes. Exceptionally in very young children, or where the vaginal entrance is very small, it is necessary to draw out the tubes to a finer calibre, although when partly filled with the bichloride solution they need only to be inserted into the ostium and the exudate washed out into their lumen.

This method is perfectly adapted to the study of structural cellular details of the exudate, which under the generic title of the "cyto-diagnosis" of urethral and vaginal discharges came into prominence some two or three years ago. Up to this time observers had largely restricted their study of these exudates merely to the detection of the gonococci. The advance of the technique of staining blood and differentiation of the various white blood cells and their constituent granules suggested a similar study in urethral and vaginal discharges. Pappenheim, Janowski, Joseph, Palano, Posner and several other observers took up the subject and tried to turn the study to practical account in diagonstic and prognostic value. They found various types of leucocytes, such as mast-cells, eosinophiles, small and large lymphocytes, globular nucleated polymorpho-nuclear leucocytes, vacuolated and other types of degenerated cells, and attempted to show that the prevalence or absence of one or another type of these various cells corresponded to some particular phase of the disease and hence was of diagnostic value. At the request of my friend, the late Dr. Robert W. Taylor, I went over this subject, not only in the male urethral inflammations in various stages, but also in vaginitis, using the various methods of blood smear technique, and feel quite sure that while the subject may be exceedingly interesting from a theoretical standpoint, it does not work out at all in practical diagnostic value, for the results are too irregular and contradictory. Thus the eosiniphiles are liable to occur in the early stages, but they occur at times equally well in the late and chronic stages and so on with various other constituents of the exudate. There are but two signs indicative of particular phases in gonorrhoea, and even these are rather vague and indefinite, namely, the presence of red blood cells, indicating a beginning stage of gonorrhoea and the appearance of considerable epithelium, mucus and the decrease of the leucocytes, showing that the process is nearing its end stages, which is a good deal like saying that the shower is over when the rain drops cease falling.

In contrasting these two methods, at Dr. Park's suggestion I followed several cases in the vaginitis wards, systematically controlling the swab smears with tube method; sometimes immediately afterwards, sometimes twenty-four hours later. Here are the results as regards the absence or presence of the gonococcus indicated respectively by the minus and plus signs.

TABLE 1

	Feb. 9, '09 Dry Swab Smears	Feb. 10, '09 Control Medicine Dropper
Clara N.	—	+
Dora A.	—	+
Dora K.	+	+
Annie K.	+	+
Sadie D.	+	+
Mary A.	+	+
Lucia	+	+
Katie M.	—	+
Caroline	+	+

TABLE 2

	Feb. 12,'09. Dry Swabs.	Moist Swabs.	Feb. 13,'09. Medicine Droppers.
Rosie G.	+	—	+
Sadie L.	+	+	+
Helen A.	—	+	+
Annie O.	+	+	+
Anna P.	—	—	+
Ella S.	+	+	+
Fannie S.	+	+	+
Mamie G.	—	—	—

These tables need no comment. It will be seen that in many instances where the swab method has given a negative decision the gonococci are nevertheless present, as shown in the smears from the medicine droppers. In table No. 1 the swabs have worked fairly well, giving positive results in 6 out of 9 cases.

In table No. 2 the suggestion of moistening the swab was carried out, giving slightly better results. These children ranged between the ages of one to six or eight years, and most of the results in each method were controlled by staining with Gram's method. It is the custom at these hospitals to examine all of the children twice a week for gonococci and all cases on admission. I have tried not to fall into the rather natural tendency of accentuating the value of a measure by exaggerating the defects of what it is designed to correct, for the swab smears have always been taken by the interne physicians of the hospitals,* and represent, I think, on an average the results obtained in hospitals in general when the method is used. Perhaps the swabs might have been taken more carefully, especially if one had in mind their imperfections, and the tables are not wholly fair, but the procedure is certainly inferior to, and less reliable than that of the tubes. This is especially to be emphasized *when the gonococci are very few in number, for they may be lost entirely in the swabs even when moistened.*

A striking instance of the value of the new method is the case of Katie L., 5 years old, admitted with scarlet fever. The swab smears were negative. While the child was still on the examination table I was astonished to find fully half of the medicine dropper tube (plus a two or three drops weak bichloride solution) filled with pus hidden in the vaginal fornices and which was reeking with gonococci. There was hardly any external evidence of the discharge and this child, if we had relied on the swab method result, would have been sent into the ward to infect others of the inmates.

Another interesting case is that of Ellen F., but 3 years old, who had been in the diphtheria ward some three weeks. This ward on the sixth floor of the Willard Parker Hospital contained 24 children. Ellen had no discharge, no signs of vaginitis, no gonococci on admission or any other time as far as could be determined, when suddenly, on March 27, 1909, a discharge developed. The swab method failed but the medicine dropper disclosed abundant pus cells with gonococci. No other child had vaginitis in the ward, and a question of great interest arises: How did Ellen develop a specific vaginitis three weeks after admission when the examination was negative and when there were no cases in this ward to infect her?

These cases are not very rare and their consideration, which is beside the issues of this paper, seems very important not only for study of gonorrhoeal infection in particular but for the role of pathogenic bacteria in general. Apparently the explanation in consonance with the theories of the present day is that such children are gonococcus carriers; that such gonococci do not provoke any pathogenic action in these particular children until some complicating congestion or light catarrh, incident to diphtheria and certain of the exanthemata provide the suitable opportunity. We hope, during the ensuing year, to see if, by an accurate method of examination of admission cases, this can be substantiated; to see whether these children belong to the class of bacteria carriers, as we have come to know them in connection with typhoid fever, diphtheria, etc.

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* As far as I can learn the swab method is quite universal in the various hospitals in New York City which contain any considerable number of children.

This investigation will not only require the washing out of the vaginal contents into the bichloride tubes, but probably also depositing the cells, before transferring to the slides, by the centrifugal machine, since the scanty collection of cells will be dispersed in a considerable volume of fluid.

A third instance of the accuracy of the tube method worth mentioning, perhaps, is that of a declining discharge of a girl of fifteen years. An unequivocal decision of the question of the gonococcus was highly important because medico-legal consideration involving the matter of criminal assault entered into the case. The physicians to whom the gonococcus determination was referred were unable to settle this question one way or the other, because smears prepared in the conventional way from absorbent cotton swabs showed such mutilation or destruction of the leucocytic cell bodies that no intracellular gonococci were found, and they declined—quite properly—to make a diagnosis on the presence of free "coffee bean" diplococci amid the variegated vaginal bacterial flora.

As a matter of fact gonococci were present but exceedingly few in number, as was disclosed by flushing out the vaginal fornices with a tube filled with a few drops 1-1000 bichloride solution. These smears showed the classical undoubtable picture groups of gonococci within the bodies of perfectly preserved leucocytes.

A tube similar to the medicine dropper might also be introduced into the meatus of the male urethra in certain cases of very slight discharge and the canal flushed out to collect the exudate for gonococcus detection.

The swab procedure is equally bad in determining the nature of inflammation or discharges from the conjunctiva. This is so very important that it needs no elaboration. Among other forms we are constantly meeting with simple catarrhal conjunctivitis in measles and scarlet fever, diphtheric conjunctivitis, and at times an inflammation accompanied by the pneumococcus, Morax, Axeufeld or Koch-weeks bacillus, but we never can be sure without the microscope, whether the case may not be one of gonorrhoeal opthalmia.

I know of at least one case of very early gonorrhoeal opthalmia in which the swab method utterly failed to show the very few gonococci present disclosed by the tube procedure. Exudates from the conjunctiva should be allowed to flow into capillary tubes. If such exudates are thick and viscous there may be drawn up into the tube a piece of attached capillary rubber tubing and expressed on the slide ana emulsified with weak bichloride solution to make the smear spread thin and uniformly. The same may be said of the examination of cases of otitis and discharges from the ear, sinuses, etc.

A trivial suggestion in the staining of the smears, although very likely gratuitous, may be of service to those having occasion to make these determinations in large numbers. The fixing of the smear may be discarded. The film can be flooded with almost any of the polychrome solutions, washed in water and examined while wet under the cover glass or afterwards dried for permanent keeping. In fact, the washing in water may be omitted and a drop or two of the polychrome solution, if not too opaque, may be allowed to flow in a capillary sheet over the film underneath the cover glass and the examination made directly. An advantage of this is, besides saving time, that the gonococci appear to swell slightly and approach more nearly their size and shape in the living condition, which is of service in comparing them with a gonococcus-like organism somewhat smaller in size.

TOXIN-ANTITOXIN MIXTURES AS IMMUNIZING AGENTS

BY

WILLIAM H. PARK, M.D., and L. W. FAMULENER, M.D.

Preliminary Note

Ehrlich early suggested that injections of diphtheria toxin partially neutralized by antitoxin would probably produce active immunity. This was demonstrated by Wernicke, Dreyer and Madsen, Morgenroth, ourselves and others. Smith, in a recent article, suggested the possible use of such mixtures in the immunization of children.

The possibility of such a practical application has suggested to us some experiments with especial reference to the safety and effectiveness of the injections.

The proportion of toxin to antitoxin in the mixture necessary to produce immunization was investigated by us in horses.

In May, 1903, we reported some experiments in which one set of horses were injected with mixtures containing toxin 0.66 of $L_{\dagger}$ dose for each unit of antitoxin and another with four times this proportion of antitoxin. Three large injections at five-day intervals each of 100,000 fatal doses of toxin produced in the first series with .66 $L_{\dagger}$ an average of 150 units per cc. of serum, in the second with .16 $L_{\dagger}$ an average of only 3 units.

Smith injected three guinea pigs with 2 units plus 1.5 $L_{\dagger}$ dose, 2 units plus 1.3 $L_{\dagger}$ dose, and 2 units plus 1 $L_{\dagger}$ dose respectively. The litters born from the first and second animals showed marked immunity at the end of 8 months. The litter from the third animal showed slight immunity at 3 months and none at 6 months.

These experiments indicate that while even a proportion of 6 units of antitoxin to 1 $L_{\dagger}$ of toxin produces slight immunity, the toxin must be in proportion of at least 1 $L_{\dagger}$ dose to 2 units to cause the production of any considerable amount of antitoxic immunity.

This brings us to the question of the safety of such mixtures. The work of Morgenroth suggests that mixtures which are toxic for one species are toxic for all. There is a difference, however, among animals as to the amount of natural immunity, therefore, of the amount of toxin required to produce the development of serious symptoms or death. Even if all guinea pigs lived, therefore, there would still be a slight uncertainty in infants.

In a series of tests we found that the least toxin which was necessary to give lasting immunity was not quite harmless in guinea pigs. Thus of four guinea pigs receiving a mixture of 1 unit plus 6/10 L_{+} dose of toxin the two larger remained permanently well, while the two smaller finally died of paralysis. Four guinea pigs receiving one-half the quantity of the same mixture all remained alive. Two other series receiving still larger quantities of the same mixture acted as the first lot. Some of these guinea pigs after two months received later two fatal doses of toxin without serious poisoning.

It is interesting to note that three of these animals later received repeated injections of toxin in increasing amounts, until finally 6,000 fatal doses were given in one injection. The blood of the animals at this time contained about from 25 to 30 antitoxin units per c.c. These experiments suggest that it is hardly safe to attempt to immunize children with toxin-antitoxin mixtures since when a degree of saturation with antitoxin is used that is absolutely safe the amount of immunity produced is small. This immunity is much less for the first two weeks, than when the same amount of antitoxin is injected without the toxin. It is possible that the toxin used in these experiments, which was produced by our culture No. 8, may have more tendency to promote late paralysis than that from other cultures.

THE COMPLEMENT BINDING TEST IN RABIES.

Jane L. Berry, M.D., Assisted by Miss A. Mann.

The work of A. Wasserman and his collaborators in the application of the complement binding test of Bordet and Gengou to the diagnosis of syphilis and other diseases has been followed, as is well known, by a long series of investigations, and many observers have confirmed the findings of Wasserman. Positive reactions are said to have been found in typhoid, tuberculosis, meningitis and many other diseased conditions. The most striking results have been those obtained in syphilitic infection.

Many views have been expressed with regard to the nature of the reaction. The original claim that specific antigens as well as anti-bodies could be demonstrated in this manner was soon abandoned, since it was proved that the test was obtained not only with specific, but also with normal extracts. The fact that alcoholic extracts were found to be effective showed the active substance to be alcohol soluble or lipoid in nature. Porges and Meier, working under Wassermann's direction, found that pure lecithin was able to bind complement with syphilitic sera, and it was evident later that a large number of other lipoids acted as well or even better than lecithin. Meantime, Landsteiner and Stankovic had obtained similar results with numerous organically suspended and colloid dissolved substances. Axamit, working with bacterial extracts, found that these alone bind complement, while Uhlenhuth obtained binding with a long list of the most unrelated and diverse materials.

None of these results have been held to affect the value of the test in the opinion of its supporters, since it is their claim that complement binding can be obtained only by the action of specific, never of normal, sera, or, if the contrary is ever true, it is said to be so rare that it can be practically disregarded. These views have not met with universal acceptance, however, since opposite results have been found by several prominent investigators.

Ranzi obtained binding of complement with normal sera alone, with extracts of tumors alone, and with tumor extracts combined with normal sera. Weil and Braun found binding to the same extent with normal as with specific sera together with tumor extracts. They considered the active agent to be not specific in nature, regarding it as probably a substance derived from degenerative cell changes in various diseased conditions. Much, testing sera from 25 cases of scarlet fever with syphilitic liver extract, found binding in 10. He obtained a similar reaction in many other diseased conditions and also with normal sera, and together with Eichelberg regarded the test as without diagnostic value.

In 1907 Heller and Tomarkin[1] sought to demonstrate, by means of the complement binding test, the existence of specific antibodies against rabies virus in the serum of animals immunized against rabies. They used the sera of rabbits inoculated subcutaneously on twenty-four consecutive days with extract of fixed virus rabbit cords made from two to eight days after death, testing the sera two to three weeks after the last injection. As test fluid they used the expressed juice of rabbit's brains obtained under pressure of 350 atmospheres, the animals having died from fixed or street virus, which were tested separately. All customary controls were used, and the experiments frequently repeated with varying amounts, but the results were negative as to diagnostic value of test in rabies. Occasionally increasing inhibition of haemolysis was obtained by the use of increasing amounts of specific immune juice and immune sera, but the same result was found upon using immune sera with expressed juice of normal brains, or normal sera together with specific or normal expressed juices.

Friedberger,[2] from a theoretical standpoint, thought this test especially well adapted for rabies. He used the serum of a horse inoculated with extract from a fixed virus rabbit brain, the serum of a rabbit immunized to horse blood, guinea pig complement, and the customary controls. He found a small amount of binding with rabies serum, but no stronger than with serum of normal horse and came to the same conclusion as Heller and Tomarkin, that no positive conclusions could be based upon this test in rabies.

A third report of complement binding tests with rabies was published after the completion of the present experiments. Baroni and his associates used three sera from animals immunized to rabies. Two were from rabbits inoculated with fixed virus rabbit cord emulsion, the first rabbit having died after six subcutaneous inoculations followed by one into the anterior chamber of the eye; the second having resisted a similar eye inoculation after one previous intravenous injection. The third serum, from Marie, was from a sheep given multiple subcutaneous injections of fixed virus at the Pasteur Institute. The three sera were tested against spleen and brain of rabbits immunized as above, with fresh guinea pig complement, according to the classical method, but, notwithstanding numerous trials no binding of complement could be demonstrated, haemolysis being always identical with that in controls of normal rabbit serum.

At the suggestion of Dr. Williams the writer undertook the present series of experiments with rabies.

The rabies material tested consisted of extracts of the brains of fixed virus rabbits, the rabbits being those furnishing the cords used in the treatment of rabies patients. The fresh brain material was crushed in a sterile tube, mixed in proportion of 1:4 with normal salt solution containing 0.5 per cent. carbolic acid, agitated at room temperature for 24 hours, and centrifuged for from one to two hours, the supernatant fluid being then drawn off into sterile flasks and placed in the ice box till needed.

Extracts were made in the same manner from the brains of normal rabbits.

A series of normal rabbits were inoculated subcutaneously with rabies material; Rabbit A, for the first few inoculations, with the above fixed virus extracts; later, this rabbit and all others of the series were injected at intervals of ten days with an emulsion of fresh fixed virus brain in salt solution, beginning with ¼ c.c., and increasing 0.2 with each treatment up to the amount of 2.5 c.c. at the last inoculation. After the fourth treatment the serum of these rabbits was used with the fixed virus brain extract for the complement binding test. Control tests were made with the serum of normal rabbits and of normal guinea pigs, and both rabies and normal sera were tested against the normal brain extract by the same method.

The blood cells used were those of the horse washed four times with salt solution, a series of rabbits having been immunized against horse blood by subcutaneous inoculations of 10 c.c. of washed horse blood cells at ten day intervals. One of these rabbits developed sufficient hemolysin after the third inoculation to dissolve 1 c.c. of a 5 per cent. suspension of horse blood cells with 1-1000 c.c. of serum. This amount gradually decreased until 1-200 c.c. of serum was required for the same amount of blood at the time of the last experiment. The amount of hemolytic serum used in each test was twice that needed for blood solution, the quantity having been ascertained by preliminary tests.

All sera were inactivated by heating at 56° for one-half hour.

Complement was furnished by fresh normal guinea pig serum.

The various substances were first diluted with normal salt solution to such strength that 1 c.c. of each could be taken for the test, making 5 c. c. in all. When one or more substances were omitted, the tubes were filled to the same level with normal salt solution. Thus diluted the fixed virus or normal extract, and fixed virus or normal serum, were measured into sterile tubes, the complement added and the tubes placed in the incubator for one hour, when the blood cells and hemolytic serum were added, the tubes returned to the incubator for two hours, then placed in the ice-box. The next morning the tubes were thoroughly shaken and again placed in the ice-box until the following morning, when the final reading was made.

The experiments were many times repeated, using sera from four fixed virus rabbits, and from two normal rabbits, together with extracts from four fixed virus rabbits and from four normal rabbits, in varying amounts and combinations, together with controls as shown in table. In this way over fifty tests were made, showing in all the same result—complete or practically complete inhibition of hemolysis. No difference whatever could be perceived between the action of normal and of fixed virus serum, except that in one series a slightly stronger binding power was shown by the normal than by the fixed virus serum. In one case the normal serum and normal brain extract were both obtained from

The following table is representative of results obtained.

TABLE I.

Sera.	*Extracts.*
Fixed Virus Rabbit.	Fixed Virus Rabbit Brain.
Normal Rabbit.	Normal Rabbit Brain.

Serum	Extract	Complement 1:10	Haem Ambocept 1:100	Blood 5%	Result
F.V. Rab., .2 c.c.	F.V. Ext., .2 c.c.	1 c.c.	1 c.c.	1 c.c.	Practically Comp. Inhibition
“ “ .1	“ “ .2	“	“	“	Nearly Complete Inhibition
“ “ .01	“ “ .2	“	“	“	Complete Haemolysis.
“ “ .006	“ “ .2	“	“	“	“ “
“ “ .005	“ “ .2	“	“	“	“ “
“ “ .2	Norm. Ext. .2	“	“	“	Practically Comp. Inhibition
“ “ .1	“ “ .2	“	“	“	Nearly Comp. Inhibition
“ “ .01	“ “ .2	“	“	“	Complete Haemolysis
“ “ .006	“ “ .2	“	“	“	“ “
“ “ .005	“ “ .2	“	“	“	“ “
Norm. Rab., .2	F.V. Ext., .2	“	“	“	Practically Comp. Inhibition
“ “ .1	“ “ .2	“	“	“	“ “ “
“ “ .01	“ “ .2	“	“	“	Complete Haemolysis
“ “ .006	“ “ .2	“	“	“	“ “
“ “ .005	“ “ .2	“	“	“	“ “
“ “ .2	Norm. Ext., .2	“	“	“	Practically Comp. Inhibition
“ “ .1	“ “ .2	“	“	“	“ “ “
“ “ .01	“ “ .2	“	“	“	Complete Haemolysis
“ “ .006	“ “ .2	“	“	“	“ “
“ “ .005	“ “ .2	“	“	“	“ “
F.V. Rab., .2	....	“	“	“	“ “
“ “ .4	....	“	“	“	“ “
Norm. Rab., .2	....	“	“	“	“ “
“ “ .4	....	“	“	“	“ “
....	F.V. Ext., .2	“	“	“	Complete Haemolysis. Occasional Sl. Inhibition
....	“ “ .4	“	“	“	Complete Haemolysis. Occasional Sl. Inhibition
....	Norm. Ext., .2	“	“	“	Complete Haemolysis. Occasional Sl. Inhibition
....	“ “ .4	“	“	“	Complete Haemolysis. Occasional Sl. Inhibition
F.V. Rab., .4	....	....	....	“	No Haemolysis
Norm. Rab., .4	....	....	....	“	“ “
....	F.V. Ext., .4	....	....	“	“ “
....	Norm. Ext., .4	....	....	“	“ “
....	....	....	1 c.c.	“	“ “
....	....	1 c.c.	....	“	Sl. Tr. Hemolysis
System Control		“	“	“	Complete Haemolysis

the same rabbit, but the result here was identical with that in all the other tests. The slight haemolytic action of normal guinea pig serum upon horse blood corpuscles, frequently noted, was not sufficiently marked to interfere with the definite comparison of results, the same being true of the slight inhibitory action sometimes seen in both fixed virus and normal extracts. Inhibition was always complete as to the action of the haemolytic serum, with invariable full hemolysis in haemolytic system controls.

These experiments agree with those of former investigators in showing the absence of any specificity for rabies in the complement binding test.

That the normal sera should have shown an equal inhibitory power to that of the fixed virus sera remains unexplained, but it is felt that errors of technique and merely accidental results are ruled out by the number of experiments made, and by the confirmatory action of the numerous controls.

The complete haemolysis obtained with all amounts of serum below 0.1 c.c. would seem to exclude precipitins here as the probable cause of complement binding.

To the above a few additional tests may be added. Serum from a normal rabbit, 0.2 and 0.4 cubic centimeters, has been brought together with extract of normal rabbit brain (from same individual), 0.2 cubic centimeter, with hemolytic amboceptor from rabbit, with fresh guinea pig complement and with sheep corpuscles. Result:—partial hemolysis (0.8) with smaller dose of serum; slight hemolysis (0.2) with larger dose. The experiment repeated, substituting extract of brain of rabbit with rabies instead of normal extract, shows slight hemolysis (0.3) with smaller dose and slight trace of hemolysis (0.1) with larger dose. The same experiments in which sera from two normal guinea pigs have been tested together in place of rabbit serum, shows complete hemolysis. There is no trace of inhibition with either extract. These results with rabbit's serum constitute the first instance in this series of tests in which any inferior binding power has been shown by normal rabbit's serum with normal brain extract, as compared with brain extracts of rabbits with rabies. In this instance an additional factor has to be taken into account, namely, the normal serum and normal brain extract have been obtained from the same animal.

An extensive bibliography upon the complement binding test is given by Fleischmann[4] and by Noguchi[5]. No other references are given here except those relating to the test with rabies.

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(2) E. Friedberger.
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Wien. Klin. Woch. 1907, XX, 879.

(3) V. Baroni, M. Cinca, C. Ionescu-Minaiesti.
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The Serum Diagnosis of Syphilis, New York, 1910.

THE INFLUENCE OF CHLORAL HYDRATE ON SERUM ANAPHYLAXIS.*

EDWIN J. BANZHAF AND L. W. FAMULENER.

In a preliminary communication read before the Society for Experimental Biology and Medicine in February, 1908, we reported the following:

"We found that by injecting a solution of chloral hydrate which was just sufficient in strength to produce hypnosis, fully 75 per cent. of all serum-sensitized guinea pigs were completely protected from a second injection of serum into the peritoneal cavity, whereas 90 per cent. of controls died. We believed that with improved technique in the dosage of chloral hydrate it would be possible to protect 90 per cent. of all fully sensitized guinea pigs. By "fully sensitized" we meant that at least three weeks or a month's time should have elapsed before the second injection of serum into guinea pigs which had received horse serum alone (1-1000 to 1-500 c. c.), at least seven or eight weeks should have elapsed before the second injection. By allowing the above interval of time to elapse, over 90 per cent. of our controls died within an hour, most of them within 20 minutes.

"We have found that the dose of chloral hydrate per gram weight of the animal was not a simple ratio; no fixed amount could be stated, much depending upon the individual idiosyncracy of the animal. Approximately, 75 milligrams of the drug to a 250-gram guinea pig, and 100 milligrams to a 300-gram guinea pig, produce the degree of hypnosis desired.

"We used a fresh 10 per cent. solution of chloral hydrate, carefully measured out the required amount into a small sterile beaker, and added an equal amount of sterile water. This diluted solution was injected into the muscles of the thigh of the animal, half into one leg and half into the other. After 20 to 30 minutes the needle was inserted into the peritoneal cavity. This caused muscular twitching and slight movement of the head. This indicated the proper degree of hypnosis. The injection of 5 c.c. serum was then given and the animal kept in a warm room. No symptoms appeared and the sleep was undisturbed. After 2½ to 3½ hours had elapsed the animal slowly recovered from the effect of the drug. No symptoms or ill effects have been observed in any of the animals. Observations have been followed for over two weeks after treatment.

"The animal, after the effects of the drug have disappeared, will react with characteristic symptoms of anaphylaxis if given a third injection of the serum. We injected only 24, 48 and 72 hours after recovery from the effects of the drug.

"If the dose of chloral hydrate has not been sufficient, the insertion of the needle into the peritoneal cavity will cause marked muscular movements, raising of the head and an attempt to regain its feet. Under these conditions, if the serum is injected the animal will die of anaphylaxis.

"On the other hand, if the animal shows no muscular twitchings whatever the dose of chloral has probably been too large. We wish to emphasize the fact that great care must be used not to overdose the sensitized guinea pig with chloral hydrate, although a sensitized as well as a normal guinea pig will recover from a large dose—considerably more than the amount mentioned above. Apparently, the combined effects of the drug and the serum in a sensitized animal produces a deeper hypnosis than the drug when given alone."

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*Read before the American Society of Biological Chemists, Baltimore, December, 1908.

Further work, following along the lines of the above preliminary report, has fully confirmed our earlier observations.

We realized that for our work it was essential to determine the best method for sensitizing the guinea pigs so that on receiving the second injection of horse serum intraperitoneally the animals would surely die from anaphylaxis. We, therefore, injected several series of guinea pigs subcutaneously with from 0.01 c.c to 0.001 c.c. of horse serum. After three to six weeks we found that in none of the series did 100 per cent deaths occur when the animals were injected with 5 c.c. horse serum intraperitoneally. In our later work we have used only those guinea pigs which had survived the routine testing of antitoxin. After a lapse of three weeks or longer, these animals, in our experience, succumb almost without exception to the second injection of 5 c.c. horse serum intraperitoneally.

In this later work, we have only given the second injection of serum to those sensitized guinea pigs which showed favorable hypnosis, indicated by only slight muscular twitchings when the needle is inserted into the peritoneal cavity. If muscular movements are marked we do not inject. In this case we allow the animal to recover from the effects of the drug, and next day increase the dose about 1/5. This usually brings about the optimum hypnosis in about twenty-five to thirty minutes. In following this method we have frequently protected 100 per cent., while 100 per cent. of the controls died.

In our work thus far it appears as though the protection chloral hydrate affords a sensitized guinea pig depends on the degree of sensitization. If we accept the hypothesis of Vaughan, this may be expressed as the amount of ferment or zymogen present in the sensitized guinea pig. Its supposed degree of activity or power in freeing or splitting off definite quantities of a poisonous substance from the serum given in the second injection accounting for the symptoms of anaphylaxis.

This appears to be the case in Table I. The eight guinea pigs were well under the influence of the drug, when the second injection was given. Numbers 393, 702 and 146 died in 15, 30 and 20 minutes respectively, with typical symptoms of anaphylaxis, the heart continuing to beat after respiration ceased.

(For Table I, see next page.)

TABLE I

INTRAMUSCULAR INJECTION OF CHLORAL HYDRATE—FOLLOWED BY 5 C.C. NORMAL HORSE SERUM

G. P. No.	Wgt.	Previous Treatment	Interval in Days	Chloral Hydrate in mg.	Second Injection Intraperitoneally	Symptoms	Results
330	310	0.56 c.c. toxin + 1/500 c.c. antitoxic horse serum	42	125	5 c.c. normal horse serum	None	Recovered
393	265	"	42	90	"	Typical	Death within 15 min.
355	260	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	28	90	"	None	Recovered
376	260	"	28	85	"	"	"
358	260	0.56 c.c. toxin + 1/350 c.c. antitoxic horse serum	32	85	"	"	"
363	280	"	32	100	"	"	"
702	395	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	62	180	"	Typical	Death within 30 min.
146	335	"	62	150	"	"	Death within 20 min.
313	395	0.56 c.c. toxin + 1/500 c.c. antitoxic horse serum	42		"	"	Death within 18 min.
372	260	"	42		"	"	Death within 25 min.
595	240	0.56 c.c. toxin + 1/200 c.c. antitoxic horse serum	28		"	"	Death within 12 min.
696	240	"	28		"	"	Death within 17 min.
513	230	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	33		"	"	Death within 12 min.
347	260	"	33		"	"	Death within 33 min.

Many series of sensitized guinea pigs were given chloral hydrate intramuscularly followed by the second injection of horse serum, intraperitoneally and in almost all cases one or two guinea pigs of each series would die with typical symptoms of anaphylaxis, even though the degree of hypnosis seemed sufficient. This leads us to believe that in such guinea pigs more poison was elaborated or split off from the serum, in a given time, than in those that were protected; for example, say, if just a single fatal dose of poison is split off the chloral will protect; but, if two or more fatal doses are split off, the chloral cannot protect the animal. A quantitative relationship may exist.

In table II the controls died promptly, while those which received the chloral were protected.

Guinea pigs No. 60 and 202 had received 180 and 160 milligrams chloral, respectively, the day before. They then weighed 410 and 370 grams. This amount, however, proved insufficient to produce the desired hypnosis. They were allowed to recover, and the following day weighed 370 and 335 grams respectively. They were again given the same amount of the drug, 180 and 160 milligrams. In twenty-five minutes favorable hypnosis was produced. The five cubic centimeters of horse serum were then injected. The following day these two guinea pigs were chloroformed and autopsied. No definite lesions were found.

Guinea pigs Nos. 240, 777, and 748, three days later, again received five cubic centimeters horse serum intraperitoneally; slight but definite symptoms appeared in the first two animals after about fifteen minutes. These symptoms subsided in about thirty minutes. In the third animal questionable symptoms were noted. Other sensitized guinea pigs that had been protected from the second injection of serum with chloral were also reinjected; about 75 per cent. showed slight but definite symptoms.

TABLE II

INTRAMUSCULAR INJECTION OF CHLORAL HYDRATE—FOLLOWED BY 5 C.C. NORMAL HORSE SERUM

G. P. No.	Wgt.	Previous Treatment	Interval in Days	Chloral Hydrate	Second Injection Intraperitoneally	Symptoms	Results
60	370	0.56 c.c. toxin + 1/350 c.c. antitoxic horse serum	85	180	5 c.c. normal horse serum	Slight twitching of ears	Recovered
202	335	0.56 c.c. toxin + 1/750 c.c. antitoxic horse serum	82	160	“	None	“
240	370	0.56 c.c. toxin + 1/400 c.c. antitoxic horse serum	74	170	“	“	“
777	325	0.56 c.c. toxin + 1/800 c.c. antitoxic horse serum	68	130	“	Slight twitching of ears	“
748	345	0.56 c.c. toxin + 1/250 c.c. antitoxic horse serum	68	155	“	“	“
438	375	0.56 c.c. toxin + 1/200 c.c. antitoxic horse serum	85		“	Typical	Death within 20 min.
143	360	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	82		“	“	Death within 13 min.
251	395	0.56 c.c. toxin + 1/250 c.c. antitoxic horse serum	81		“	“	Death within 20 min.
256	360	0.56 c.c. toxin + 1/750 c.c. antitoxic horse serum	78		“	“	Death within 32 min.
25	290	0.56 c.c. toxin + 1/900 c.c. antitoxic horse serum	50		“	“	Death within 25 min.

CHLORAL HYDRATE INJECTED INTRAMUSCULARLY, FOLLOWED BY NORMAL HORSE SERUM INTRACARDIACALLY.

Sensitized guinea pigs were given chloral hydrate in the same manner mentioned above. Those animals which showed favorable hypnosis were injected directly into the heart with ¼ cubic centimeter of normal horse serum. After about one-half minute all the animals showed typical symptoms of anaphylaxis, dying within three to four minutes. All the controls also died within three to four minutes. This experiment was repeated several times with the same results, none of the guinea pigs were protected.

All these animals were autopsied. We found no deaths due to direct hemorrhage. Gross lesions were found in other organs.

TABLE III

INTRAMUSCULAR INJECTIONS OF CHLORAL HYDRATE—FOLLOWED BY NORMAL HORSE SERUM INTRACARDIACALLY

G. P. No.	Weight	Previous Treatment	Interval in Days	Chloral Hydrate in mg. Intramuscularly	Second Injection Horse Serum Intracardiacally	Symptoms	Result	Autopsy
353	290	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	21	125	¼ c.c.	Typical	Death within 3 min.	No Haemorrhage in Pericardium
535	330	0.56 c.c. toxin + 1/400 c.c. antitoxic horse serum	51	160	"	"	Death within 4 min.	"
107	300	"	20	140	"	"	"	"
237	280	"	20	120	"	"	"	"
218	520	0.56 c.c. toxin + 1/100 c.c. antitoxic horse serum	29		" "	"	Death within 3 min.	"
272	300	0.56 c.c. toxin + 1/400 c.c. antitoxic horse serum	20		"	"	"	"
100	275	"	20		"	"	Death within 4 min.	"

In seeking an explanation for these results we came to the conclusion that a certain optimum content of the drug in the circulation was essential to protect the animal; that possibly the lipoids played an important role in anaphylaxis; that if we injected a sufficient amount of chloral hydrate directly into the circulation we would obtain a loose physico-chemical combination with the vitally important lipoids of the cells and in so doing change their normal relationship to the other cell constituents, through which an inhibition of the entire cell chemism would result. With this in mind, we injected sensitized guinea pigs weighing between 275 to 300 grams with 30 milligrams of the drug directly into the heart. Even before completion of the injection the animals were under the influence of the drug. After two to four minutes the dose was repeated again into the heart. After again allowing two to four minutes to elapse ¼ cubic centimeter of horse serum was injected into the heart.

Chloral hydrate given in this manner protects about 75 per cent. of the sensitized guinea pigs from the second injection when made directly into the heart.

Chloral Hydrate Injected Intracardiacally, Followed by Normal Horse Serum.

Solutions of chloral hydrate were given intracardiacally in divided doses to sensitized guinea pigs. When complete hypnosis was produced ¼ cubic centimeter of normal horse serum was given in the same manner.

The dilutions for injection were such that not more than 1½ cubic centimeters were given at one time. These injections were given very slowly, so that the danger from possible dilatation of the heart could be largely ruled out. Also, since intracardiac injections very frequently result in injury to the heart muscle, causing hemorrhages, and since the whole dose does not always enter the heart, experiments were repeated many times. Autopsies were performed in all cases in which death occurred in order to exclude mechanical injury with resulting hemorrhage as the cause of death.

In our preliminary work the selected dose of the drug, which was about one-half of the amount for an intramuscular dose, was given intracardiacally at one injection. This, however, frequently caused the death of the animal. We, therefore, resorted to divided doses at short intervals. With this method few deaths resulted from chloral injections.

In table No. IV, guinea pig No. 139 received 45 milligrams chloral hydrate intracardiacally; even before the injection was completed the animal was under the influence of the drug. The respiration was deep and irregular for about ten seconds, then short and rapid for about one minute. Three minutes after the first injection 40 milligrams more of the drug were given; the same irregular respirations were observed. Two minutes after the second injection of the drug ¼ cubic centimeter normal horse serum was given. A few twitchings at the nose were noticed. Thirty minutes later the animal showed signs of recovery from the effects of the drug and 2½ hours later complete recovery was effected. Guinea pig No. 995 received 40 milligrams chloral hydrate. The animal was under the influence of the drug at the completion of the injection. The respiration was deep and iregular for about ten seconds, then short and rapid. Two minutes after the first injection 40 milligrams more of the drug were given. Two minutes later ¼ cubic centimeter normal horse serum was given. No symptoms were noticeable. About 2½ hours later the animal had recovered from the effects of the drug.

Guinea pig No. 16 received 35 milligrams chloral hydrate. The animal was under the influence of the drug at the completion of the injection. Four minutes later 30 milligrams more of the drug were given. Three minutes later ¼ cubic centimeter of normal horse serum was given. No symptoms were noticeable. About two hours later the animal had recovered from the effects of the drug.

Guinea pig No. 8 struggled considerably while receiving the 30 milligrams of the chloral hydrate, so that most of the drug did not enter the heart. The animal could run around immediately after the injection. Four minutes later the animal was still walking around. We then gave it 35 milligrams more of the drug in the heart this time. The animal was then under the influence of the drug. Four minutes later ¼ cubic centimeter normal horse serum was injected. About one-half minute later typical symptoms developed, the animal dying within four minutes. Autopsy showed no direct hemorrhage. It appears in this case as though an insufficient amount of chloral had entered the circulation.

Guinea pig No. 24 struggled considerably while receiving the 30 milligrams of chloral. Most of the drug did not enter the heart. The animal could run around. Having in mind the result of the inoculations of guinea pig No. 8, we decided to wait twenty minutes before injecting again. At the end of twenty minutes the animal was in a partial stupor. Thirty milligrams of chloral were given, the animal being under the influence of the drug at the completion of the injection. Four minutes later an additional 30 milligrams of the drug were given.

This was followed three minutes later with ¼ cubic centimeter normal horse serum. No symptoms were noticeable. About three hours later the animal recovered from the effects of the drug.

Twenty-four hours later guinea pigs Nos. 139, 995, 16 and 24 received an additional ½ cubic centimeter normal horse serum intracardiacally. All showed typical symptoms after about ½ minute. Respiration ceased within three minutes. The heart continued to beat after respiration had ceased. Autopsies showed none of the deaths due to direct hemorrhage. These experiments were repeated many times with similar results.

TABLE IV

INTRACARDIAC INJECTIONS OF CHLORAL HYDRATE—FOLLOWED BY ¼ C.C. NORMAL HORSE SERUM. TIME INTERVAL BETWEEN INJECTIONS 2 TO 4 MINUTES

G. P No.	Weight	Previous Treatment	Interval in Days	Chloral Hydrate in mg. Intracardiacally	Second Injection Horse Serum Intracardiacally	Symptoms	Result	Autopsy
139	380	0.73 c.c. toxin + 1/500.c c antitoxic horse serum	50	1st inj. 45 mg. 2d inj. 40 mg.	¼ c.c.	A few scratches at nose	Recovered	
995	340	0.73 c.c. toxin + 1/1200 c.c. antitoxic horse serum	30	1st inj. 40 mg. 2d inj. 40 mg.	"	None	"	
16	310	0.73 c.c. toxin + 1/100 c.c. antitoxic horse serum	50	1st inj. 35 mg.	"	"	"	
8	335	0.73 c.c. toxin + 1/600 c.c. antitoxic horse serum	50	1st inj. 30 mg. missed heart 2d inj. 35 mg.	"	Typical	Death within 4 min.	No hemorrhage in pericardium
24	305	0.73 c.c. toxin + 1/450 c.c. antitoxic horse serum	30	1st inj. 30 mg. missed heart 2d inj. 30 mg. 3d inj. 30 mg.	"	None	Recovered	
13	300	0.73 c.c. toxin + 1/500 c.c. antitoxic horse serum	50		"	Typical	Death within 3 min.	No hemorrhage in pericardium
42	320	0.73 c.c. toxin + 1/1250 c.c. antitoxic horse serum	30		"	"	"	"
33	360	0.73 c.c. toxin + 1/400 c.c. antitoxic horse serum	30		"	"	"	"

The fact that those sensitized guinea pigs which, while under the influence of the drug, were protected from the second injection of serum are still sensitized, leads us to the following conclusions: Certain parts of the serum necessary to cause anaphylaxis when injected into the circulation of a sensitized pig, must be rapidly destroyed or eliminated from the circulation. Or, these parts may combine with cells not vitally important to the immediate life of the animal. If this be the case the ferment or zymogen in those protected, sensitized guinea pigs has not all been utilized in destroying or splitting the serum.

In other words, the animal is not in most cases vaccinated, but will react with typical symptoms, followed by death, if, after recovery from the effects of the drug, an additional ½ c.c. of horse serum is given intracardiacally.

We wish to emphasize the fact that from our experiments a certain optimum content of the drug in the circulation is essential to protect the animal. The drug is rapidly destroyed or eliminated from the circulation. This we believe can be shown by allowing twelve to fifteen minutes to elapse between the intracardiac injection of the drug and twelve to fifteen minutes to elapse before injecting the serum. Chloral hydrate given in this manner, with this time allowance will not protect sensitized guinea pigs from the second injection intracardiacally.

TABLE V

INTERCARDIAC INJECTIONS OF CHLORAL HYDRATE—FOLLOWED BY ¼ C.C. NORMAL HORSE SERUM. TIME INTERVAL BETWEEN INJECTIONS 12 TO 15 MINUTES

G. P. No.	Weight	Previous Treatment	Interval in Days	Chloral Hydrate Intracardiacally	Second Inj. Horse Serum Intracardiacally	Symptoms	Result	Autopsy
168	300	0.73 c.c. toxin — 1/1400 c.c. antitoxic horse serum	18	1st inj. 30 mg. 2d inj. 30 mg. 3d inj. 30 mg.	¼ c.c.	Typical	Death within 5 min.	No Haemorrhage in Pericardium
11	290	0.73 c.c. toxin — 1/1700 c.c. antitoxic horse serum	18	1st inj. 30 mg. 2d inj. 30 mg. 3d inj. 20 mg.	¼ c.c.	"	"	"

CHLORAL HYDRATE INJECTED INTRACARDIACALLY, FOLLOWED BY ¼ C.C. NORMAL HORSE SERUM INTRACEREBRALLY.

Sensitized guinea pigs were trephined and then given divided doses of chloral hydrate in the manner mentioned above. The time allowance between injection of the drug was from two to four minutes. One-quarter cubic centimeter of normal horse serum was then given intracerebrally after the method of Besredka. About 75 per cent. of the sensitized guinea pigs tested in this manner were protected from the second injection intracerebrally.

TABLE VI

INTERCARDIAC INJECTIONS OF CHLORAL HYDRATE—FOLLOWED BY ¼ C.C. NORMAL HORSE SERUM INTRACEREBRALLY. TIME INTERVAL BETWEEN INJECTIONS 2 TO 4 MINUTES

G. P. No.	Wgt.	Previous Treatment	Interval in Days	Chloral Hydrate Intracardiacally	Second Injection Horse Serum Intracerebrally	Symptoms	Result
43	400	0.73 c.c. toxin + 1/750 c.c. antitoxic horse serum	22	1st inj. 45 mg 2d inj. 45 mg.	¼ c.c.	None	Recovered
2	395	0.73 c.c. toxin + 1/500 c.c. antitoxic horse serum	32	1st inj. 40 mg. 2d inj. 40 mg.	"	"	"
26	295	0.73 c.c. toxin + 1/400 c.c. antitoxic horse serum	37	1st inj. 35 mg. 2d inj. 35 mg.	"	"	"
7	415	0.73 c.c. toxin + 1/500 c.c. antitoxic horse serum	32	1st inj. 45 mg. 2d inj. 45 mg.	"	"	"
29	275	0.73 c.c. toxin + 1/300 c.c. antitoxic horse serum	40		"	Typical	Death within 5 min.
333	340	"	32		"	"	"
934	380	"	38		"	"	"

With the different methods of administering the second injection of horse serum into sensitized guinea pigs, which were favorably under the influence of chloral hydrate, we were able to protect about 75 per cent. In our preliminary communication we stated that we believed that with improved technique in the dosage of chloral hydrate it would be possible to protect 90 per cent. of all fully sensitized guinea pigs.

Now, however, we have come to the conclusion that, if the smallest dose of serum which will just kill any animal of a given series of sensitized guinea pigs regularly, be injected into the remainder of the animals of the same series of sensitized guinea pigs, when properly under the influence of chloral hydrate, that protection will be afforded in practically all cases.

AN OCCASIONAL COMPLICATION OF THE PASTEUR ANTIRABIC TREATMENT

By D. W. Poor, M.D.

Since the Pasteur antirabic treatment has come into general use cases of obscure illness have been from time to time reported during the course of the treatment, which in a few rare instances have ended in death. Some of these were undoubtedly intercurrent conditions not connected with the antirabic treatment. Others may have been due to bacterial contamination, but in still others there is sufficient similarity in the symptoms to awaken the suspicion that they may have been due to the treatment itself. The older writers considered such illness to be due to the original canine infection modified by the Pasteur injections, or even to a form of rabies produced by the injections themselves. The manifold symptoms due to neurasthenia and hysteria occasionally appearing in those taking the Pasteur treatment have still further complicated the subject so that it is not altogether surprising that one reads that the "Pasteur treatment may produce blood poisoning, neuritis, ascending paralysis, insanity and even rabies itself."

It was not until 1905 that an extensive investigation was made of this subject. In that year Remlinger published an article in the Annales de l'Institut Pasteur on paralytic accidents occurring during the course of antirabic treatment. The article was the result of an investigation among the various European laboratories and institutes, following a case which occurred in his own practice. The record of Remlinger's case is as follows:

A boy 13 years old, bitten on the right thigh by a dog diagnosed rabid by a veterinary surgeon. Treatment was begun a week later. On the morning of the twelfth day of treatment he complained of severe pain in the muscles and joints, and a sensation of great weakness in the legs. He attributed the symptoms to a cold shower bath taken the night before. The injections were continued. The next day the pain was no better, there was complete paralysis of the legs, with retention of urine, necessitating catheterization. The antirabic treatment was suspended.

There was no fever. The following day his condition was worse. The pain extended to the neck and face, and paralysis began in the arms, The fourth day the paralysis of the arms was more pronounced. During the next week the pain diminished, and then disappeared. The tactile sensibility was preserved, but reflexes abolished. Retention of urine and constipation persisted. The paralysis of legs was still complete, that of the arms incomplete. On the thirteenth day movement slowly returned to the arms, but the paralysis of the legs remained. Retention of urine was replaced by incontinence. A week later the patient could move the great toe of the left foot. The following day he could move all the toes of the left foot. Later he moved the left leg and then was able to move the great toe of the right foot. In another week the patient was able to raise himself and take a few steps. From this time convalescence was rapid and he left the hospital thirty-eight days after the onset of his illness. There remained a little anemia and weakness, but no muscular atrophy.

Remlinger has collected the records of about 40 cases which showed distinct objective symptoms. These occurred among a total of approximately 100,000 persons treated. Two were fatal, one after symptoms lasting one month and one after nine months. Analysis of the parts of the body involved is shown in the following table:

Legs and sphincters	17	cases
Legs alone	6	“
Legs, sphincters, arms, face	4	“
Legs, sphincters, face	4	“
Arms and face	1	case
Arms alone	1	“
Face alone	1	“
Legs, arms, sphincters and bulbar symptoms	1	“
Legs and face	1	“
Legs and sphincters and bulbar symptoms	1	“

A résumé of the symptoms as given by Remlinger is as follows:

The onset may be from the eighth day of treatment to the end of the first week following it. There is usually more or less paralysis of the legs with or without involvement of the bladder and rectum. These symptoms are frequently preceded by lancinating pains in the limbs and occipital headaches. The sensory symptoms are variable. There may be hypersensibility of the skin with increase of reflexes or anesthesia with loss of reflexes. Various forms of paresthesia are apt to occur. The symptoms may be confined to the legs or assume the type of an ascending paralysis. The muscles of the face may be involved and bulbar paralysis with symptoms of dyspnoea, tachycardia and dysphagea may occur. Even in these last cases recovery is possible. In the majority of cases, after about three weeks the symptoms ameliorate and recovery is complete in several days or weeks. One case lasted six months and one is reported to have been unimproved at the end of six years. Apparently the continuation of the treatment has no effect upon the course of the condition.

In 1908 Remlinger[1] reports two more cases, Masson[2] one case, Pampoukis[3] [4] three cases, and Babes[5] and Mironescue report a fatal case. The history of the latter is as follows:

A woman 40 years of age, thin and of nervous temperament, was severely bitten by a rabid dog and began the treatment six days later. After 14 days of treatment of medium intensity, the course was interrupted by the appearance of paralysis of the legs without hydrophobia. The paralysis took on the character of the ascending type of Landry. The patient was sent to the hospital, where she died the next day. The autopsy showed lesions affecting mainly the lumbar cord. There were areas of softening and hemorrhage, involving both the gray and white matter.

REPORT ON ADDITIONAL CASES

Out of a total of 2,300 patients treated I have encountered twelve cases presenting this interesting complication. I have included those which gave symptoms suggesting a mild neuritis as well as those which seemed to indicate an involvement of the cord, as the process appears to be identical in all, and merely variable in degree.

Unfortunately it has been impossible to learn the subsequent history of most of these cases.

(1) A. E., male, 78 years of age, received two small wounds of the wrist from a dog in which the diagnosis was uncertain. Treatment was sent* for 21 days. During the last two or three days of treatment and

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*Virus prepared at the laboratory and sent by mail to the attending physician, who carries out the treatment.

for the next two weeks patient had numbness and weakness in the upper and lower extremities.

(2) S. McN., male, aged 33, severely bitten on the hand by a rabid dog. Treatment sent for 22 days.* On the seventeenth day of treatment there were pains and other symptoms of neuritis in the left leg. This was followed by retention of urine for several days, necessitating catheterization. Patient confined to bed. At the end of 15 days the symptoms had about disappeared.

(3) H. A., male, 25 years old. Scratched by a cat in which the diagnosis was uncertain. Treatment sent* for 18 days. During the last six days there was severe pain in the sacral region. Patient failed to report further.

(4) J. B. M., female, 38 years old. Scratched on the hand, which it was thought might have been infected by the saliva of a mad dog. Treatment sent* from September 8 to September 28. On October 9 the attending doctor reports that for the past few days patient has had pain and numbness in the extremities. No further history obtainable.

(5) W. P., male, 42 years old. Four or five superficial wounds on hands inflicted by dog with clinical symptoms of rabies. Treated 21 days; the last part of the treatment sent;* the doctor reports patient has severe neuralgia, especially in the legs, for the two weeks following treatment. Duration unknown, as further information was not received.

(6) Mrs. B., 42 years old. A slight wound came in contact with the saliva of a rabid dog. Patient treated 15 days. Paresthesia, general depression and pains throughout body accompanied by marked general depression. These symptoms were said to have lasted a year, during part of which time patient was under treatment at a sanitarium. The history of this case is somewhat complicated by the onset of the menopause during this period.

(7) Mr. S., aged 52, and daughter, aged 17. Wounds on fingers, fairly severe. Diagnosis of dog doubtful. Treatment sent* for 14 days and then stopped on account of the following symptoms, especially pronounced in Mr. S.: There were severe headache, pains in legs and

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*Virus prepared at the laboratory and sent by mail to the attending physician, who carries out the treatment.

arms, and profound general weakness. About 20 months later the attending physician writes as follows: "Mr. S. states that five weeks after receiving the last injection he had numbness and swelling of the right hand, the numbness especially along the distribution of ulnar nerve. Symptoms disappeared in about two months. A numb sensation along the outer aspect of the left thumb has persisted until the present time. He complains of pain between the shoulders extending up to the right occipital region. Patient further states that for about two months after taking treatment he had partial paralysis of right wrist, extension being difficult. Even at the present time he is frequently troubled with sciatic pains. All of these symptoms appear to be abating during the past few months.

"Miss S. had no numbness, but the pain in the dorsal region, the occipital and sciatic pains were similar to her father's. During the past three months, however, she has been free from pain. At the time of treatment she weighed 90 pounds. At the present time she weighs 115 pounds, and appears to be in perfect health. I think Mr. S. is somewhat of a neurasthenic."

(8) J. R., male, 50 years old. Two scratches on the hands which it was thought might have been infected with the saliva of a rabid dog. Treatment sent* for 17 days. On fifteenth day of treatment patient complained of numbness in both thighs and legs, mainly the left, which lasted three weeks.

(9) Name unknown. Coachman. Wounds exposed to the saliva of a rabid dog. Tretament sent* for 18 days. During the latter part of the course patient complained of gastric indigestion, and numbness in the extremities. Duration and further history unknown.

(10) R. T., 38, male. Very slightly bitten on the hand by a dog in which the diagnosis was doubtful. Treated at the laboratory 15 days when patient complained of severe pain in back and down both thighs. Treatment stopped. On examination three days later patient was found to be suffering from severe lumbar and sciatic pain which at night prevented sleep. There was slight difficulty in walking, which was ap-

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*Virus prepared at the laboratory and sent by mail to the attending physician, who carries out the treatment.

parently due to the pain. No distinct signs of paralysis noted. Skin sensations and knee jerks normal. No bladder and rectum involvement. Pronounced occipital headache. Later the pains and paresthesia extended to arms and face. Insomnia continued troublesome. Patient exhibited a variety of neurasthenic symptoms and finally went to a sanitarium. Recovery after several months.

(11) C. W., aged 4. Badly bitten on face by rabid dog. Treatment sent 11 days later. There is some question as to the nature of this case, but it is classified with the others for want of a better understanding of it. After 17 days of treatment child was sent to the hospital suffering with severe headache and marked restlessness. Temperature 99.6, pulse 96. The following day there were dysphagia, difficulty in articulation, marked twitching of muscles of face, severe pain in the back of the neck and marked constipation. Patient seemed very rigid. Third day the patient was quieter, but apparently unable to speak. Temperature 101.8. Urinalysis negative. Fourth day a small abdominal abscess was opened. Headache continued. Urination involuntary. Later in the day the twitching disappeared, and patient relaxed. Fifth day twitching returned, urine still passed involuntarily. Sixth day patient was more comfortable, but there was occasional twitching. Seventh day, temperature 99.4. Twitching had stopped. Nine days later patient discharged, well.

(12) H. K., male, aged 63. Bitten quite severely on the arm by a dog which, though sick, was shown later not to have been rabid. Patient was a large, well preserved man. Previous history unimportant. Treatment began five days after the bite and continued for 12 days at the laboratory. Although patient's wife stated later that he had complained of certain symptoms almost from the beginning, he made no complaint at the laboratory, and on the day of the last injection he seemed normal. The following report was sent by the family physician:

"After the second injection patient began to complain of general weakness, pain at the points of injection and restlessness at night. His complexion seemed sallow and he tired easily after slight exertion. On the day after the twelfth injection he walked, with the aid of a cane, to my office, complaining of digestive disturbance, particularly flatulence.

He also complained of numbness in the legs. That same night I was summoned to the patient's house and found him sitting upright in a chair with anxious countenance, marked restlessness and loss of strength. The pains and paresthesia were about the same as in the morning. He was then able to swallow liquids, but solids, such as bread, caused paroxysms of cough. There was no fear of water. A most pronounced change since morning was the development of a profound prostration. In spite of veronal and trional he passed a very restless night, appearing the next morning very weak. He was unable to sit up in bed and could speak only in a whisper. Examination of the throat was negative. Tongue was protruded without difficulty. Temperature by mouth was 96, pulse about 70, and of good quality. At 10 A. M. patient was given a small sip of water which produced violent cough, dyspnoea and cyanosis. Pulse during the attack 66, and of good quality. At 2 P. M. his condition was still more grave and a consulting physician was called. During the examination there developed extreme dyspnoea, a weak, ineffectual cough, great restlessness and air hunger. Patient constantly threw himself about. Expression extremely anxious. About 10 minutes later respiratory paralysis set in, the heart continuing to beat about two minutes longer. Its action at first was quite strong and regular, gradually becoming slow (about 18 per minute) and weaker. Death occurred at 3.10 P. M.

Practically no changes were found in the brain and cervical cord of this case either in the gross inspection or microscopic examination of sections. No examination was made of the lower portion of the cord. The body was injected with an embalming fluid containing formalin, before autopsy. A portion of the brain apparently unaffected by the fluid was emulsified and injected into guinea pigs without result.

Cause of the Symptoms.

The various hypotheses which have been advanced to account for these symptoms are:

(1) Bacterial contamination, (2) hysteria, (3) modified canine rabies, (4) rabies due to the treatment, (5) rabic toxin.

Bacterial contamination may be dismissed as a cause for the reasons that cultures of the emulsions do not show the presence of bacteria, moreover, if this was the cause, more individuals would probably be affected at the same time, and further, there would not be the almost uniform tendency to recovery.

Hysteria is out of the question. In one of our cases, the course of the illness was somewhat modified by symptoms of hysteria, but these set in after the characteristic neuritis. In none of the other recorded cases were symptoms of hysteria present. Furthermore, if due to hysteria the symptoms would not be of so uniform a character. Neither alcohol nor syphilis can be held accountable. All of our cases were in excellent physical condition.

Modified canine rabies may be excluded since in quite a number of instances the animal inflicting the bite was shown not to be rabid.

Rabies due to the treatment is equally unsatisfactory as an explanation, since the symptoms often set in even before they would after subdural infection with fixed virus. Further, the brain substance of those cases going to autopsy has been incapable of producing rabies. Babes has been able to produce similar paralysis in animals by the injection of heated virus in which the rabic organism is dead. He has also noticed these symptoms in a man who had received only virus which was heated to 75° or 80° and cords dried no shorter time than eight days.

I have observed the death of guinea pigs injected with virus killed by heat as well as that killed by chloroform. The paralyses noted by Babes were not observed. Nothing of importance was seen at autopsy. The brain substance was incapable of producing rabies. An interesting fact is that, as in humans, only one or two out of a group of seven or eight pigs injected would be affected.

The most plausible theory thus far advanced is that the symptoms are due to the presence of a toxin in the emulsion, this toxin being characteristic of the rabic virus. The presence of this toxin together with an idiosyncrasy on the part of the patient is held accountable for the symptoms by Babes and Remlinger.

Pampoukis, while accepting the toxin part of the theory, offers as a substitute for "idiosyncrasy" the following idea: The rabies toxin is taken up by the phagocytes and so changed by them in a biochemical way that it can no longer act on the nervous system. When, however, the organism is so weakened that the phagocytes cannot perform their function, the toxin reaches the nervous system unchanged and so irritates it as to produce the symptoms. According to him the most important cause of this "upsetting of equilibrium" in the body is chilling of the patient, especially by cold bathing. In six of the 40 cases recorded by Remlinger cold bathing immediately preceded the onset of symptoms. In the two cases reported by Pampoukis paralysis followed a cold bath in one, and chilling of the body in the other.

No attempt has been made to seek for this factor in the etiology of our cases, although it is recalled that one of those having most pronounced symptoms was accustomed to swimming long distances in the sea during the period of treatment.

Mariensco supposes that the cause may be a cytotoxin due to the presence of a foreign protein and intimates that the symptoms would follow the injection of normal nerve substance. I have been unable to find any animal experiments in literature which directly prove this.

On the other hand, Babes has injected not only animals but persons with large amounts of normal nervous substance without producing any paralysis. In a series of sixteen guinea pigs which I injected intraperitoneally with large amounts of emulsion of dog brain for the production of neurolytic serum, I saw no bad effects whatever. Babes does not believe that the amount and strength of the virus are factors because he has not noted the symptoms in those taking the strongest treatment. A more convincing argument would have been produced if a detailed analysis of his cases had been given, especially with reference to the proportion of severe and mild cases treated.

It seems to be generally admitted that these paralytic accidents do not accompany the dilution method of Hogyes, in which very small amounts of nervous matter are injected.

I feel that while idiosyncrasy is a most important factor, the character of the virus is also of importance.

One of our cases occurred in 1906, four in 1907 and seven occurred during the nine months between February and October, 1908. During the succeeding nine months there have been no cases. It is a coincidence that may have some bearing, that about October, 1908, we began to inoculate rabbits from the cord rather than from the brain of the rabbit of the preceding series. In this way we undoubtedly used a weaker virus, as the rabbits did not show the initial symptoms until a day and sometimes two days later than by the other method. During this time also we have been careful to give smaller and weaker doses to those who were but slightly bitten.

It is a curious fact that out of our 12 cases nine were either but very slightly bitten, or only had wounds exposed to the saliva of rabid dogs, or else were bitten by animals proved not to be rabid. Only two were severely bitten by rabid animals, and in one the diagnosis was unknown.

In conclusion it may be said:

(1) that in a small proportion of patients undergoing Pasteur antirabic treatment, a complicating affection of the nervous system occurs, the symptoms of which vary from what is apparently a mild neuritis to an acute ascending paralysis. While the prognosis is almost always good, a certain small undeterminable proportion of the cases terminates fatally.

(2) That these symptoms are probably due to substances in the nature of toxins associated with the rabic virus.

(3) That in the absence of a clear understanding of these cases the most important predisposing condition must be regarded as personal idiosyncrasy.

(4) That exposure to cold and especially cold bathing appears to be an associated causative factor.

(5) That the variations in the virus (strength, etc.) probably are of importance in the etiology.

(6) That while these complications should not be considered in the case of those who evidently need the Pasteur antirabic treatment, account should be taken of them in deciding on the advisability of this treatment for those in whom infection is doubtful.

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THE IMMUNIZING PROPERTIES OF KILLED RABIES VIRUS

D. W. Poor, M.D.

The action of dead rabies virus in producing immunity is of interest not only from a practical but from a theoretical standpoint. A consideration of the best methods for combating infection in man as well as the practicability of vaccinating dogs against rabies depends to a considerable extent on the availability of dead virus for this purpose.

The bearing of this question on the theoretical considerations regarding the nature of rabies infection is ably discussed by W. F. Harvey and Anderson McKendrick in their work on the theory and practice of antirabic immunization.[1] These authors conclude that the immunizing property of dried cords is directly proportional to their infectivity and inversely proportional to their loss of water, and that, therefore, cords dried nine days and more are practically useless for immunizing purposes.

On the other hand, Fermi[2] as the result of his experiments recommends the use of dead virus only, his plan being to kill the virus with carbolic acid and store the emulsions for future use.

Otto Heller in his work on protective inoculation against rabies, Jena, 1906, gives the experience of others in the use of virus killed in various ways, e. g., heat and by glycerin, and his own experiments in the use of virus killed by grinding according to the method of McFadyen. The brains of rabbits dead with fixed virus infection were hardened by freezing with liquid air. They were then ground up in a metallic mortar operated by a motor, for several hours. The mortar with the brain material was kept cold during the operation by exposure to liquid air. He found that the virus was killed by three hours grinding but not by one and one-half hours grinding.

He treated ten rabbits by five or six subcutaneous injections of this virus, the injections being given several days apart. Six of the animals survived, one died of rabies and three from other causes. All the controls died of rabies.

It is a rather curious point that in the treatment of the rabbits which varied slightly in detail in each case, at least one injection of material ground for only two hours was given in each instance. According to his previous experiments it is uncertain whether or not this was killed.

Heller's conclusions are (1) The live virus is not absolutely essential for the production of immunity. (2) McFadyen's method is the best, as it destroys the organisms without changing their action. (3) It is possible to produce paralysis and marasmus (toxic symptoms) by means of killed virus.

The following tests were made, to determine to what extent immunity could be produced by virus killed in various ways and to gain some idea of the length of time each virus would preserve its immunizing properties.

Three kinds of virus were used. First, an emulsion in salt solution prepared from the brain of a fixed virus rabbit. This emulsion was killed by heating to 60° C. for twenty minutes. The immunizing strength of this emulsion was tested in the fresh state and after having been kept in the ice-box at 45° F. for a week. Second, emulsions made from the dried cords of the ordinary Pasteur series. Cords dried twelve, eleven, ten and nine, eight and seven days were used. In another experiment similar cords with the exception of the eight and seven day cords were used. Third, the brains of fixed virus rabbits were spread in thin films and the virus dried and at the same time killed by exposing it to dry heat at 50 to 53 and 60 to 63 as shown in the following table. This virus was stored in the ice-box before being used in one case for eleven days, in the second test for two and one-half months. Emulsions were then made in salt solution as in the ordinary Pasteur method.

In these tests the guinea pigs were first treated for varying lengths of time, ten to twenty days. At the completion of the series of injections (two c.c. each day) a period of rest from six to sixteen days was allowed for the development of possible immunity. At the end of this time, each guinea pig of the series, together with an equal number of controls, was given from one to one and a half cubic centimeters of a thick emulsion of street virus in the region of the right sciatic nerve.

The following table shows the results of nine tests upon guinea pigs treated with these three forms of killed virus. (See page 134.)

It appears, as would be expected, that in a general way the mortality in the treated animals varies according as they are treated for a longer or shorter time, according to the severity of the infection by the street virus as shown by the mortality and incubation of the control pigs, and according to the length of time allowed for the development of immunity.

It may be said that the results shown in the table indicate.

(1) What antirabic immunity may be produced by these three varieties of killed virus.

(2) That the "Pasteur virus" gives a better immunity than the brain virus emulsions heated to 60°.

(3) That this latter virus loses much of its immunizing power by being kept at 45° F. for a week.

(4) That by drying the fixed virus brain and at the same time killing it by heat, a virus is obtained which, when kept in the dry state at 45° F. for two months, has still a very considerable immunizing power.

It may be added that the minimum degree of heat required to kill the brain virus, as used in the method described above, has not yet been determined. Exposure to from 50 to 53° C. for eighteen hours kills and dries the virus. Exposure to 36° C. for twenty-four hours dries but does not kill it. Emulsions made from this latter virus kill guinea pigs in 6-7 days when inoculated subdurally.

We are at present preparing the killed and dried virus from the brains of our fixed virus rabbits, and this will be tested on a series of dogs with both before and after infection.

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HEATED VIRUS EMULSION FRESHLY PREPARED

Test Number	No. of pigs Treated	Length of Treatment	Amount of Infecting Virus and Time at Which It Was given	Mortality and Average Incubation of Treated Animals		Mortality and Average Incubation of Controls
1	7	20 days	1 c.c. of street virus 16 days after end of treatment	16% 29 days		85% 16 days
2	7	13 days	1½ c.c. 6 days after end of treatment	42% 27 days		85% 16 days
3	7	12 days	1½ c.c. 11 days after end of treatment	85% 18 days		100% 13 2/7 days

HEATED VIRUS EMULSION STORED FOR ONE WEEK AT 45 DEG. F. BEFORE USE

4	7	13 days	1½ c.c. 6 days after end of treatment	57% 14 days		85% 16 days
5	6	13 days	1 c.c. 11 days after end of treatment	83% 17 3/5 days		100% 15 2/3 days

PASTEUR VIRUS

Test Number	No. of Pigs Treated	Length of Treatment	Virus Used	Amount of Infecting Virus and Time at Which It Was Given	Mortality	Mortality and Average Incubation of Controls
6	7	15 days	Cords dried 12-7 days	1 c.c. 15 days after end of treatment	0	100% 18 5/7 days
7	6	12 days	Cords dried 12-9 days	1 c.c. 11 days after end of treatment	0	100% 15 2/3 days

BRAIN VIRUS, HEATED, DRIED AND STORED

8	5	12 days	Brain virus heated for 18 hrs. between 50 and 53 deg. C. and stored 11 days	1½ c.c. 11 days after end of treatment	0	100% 13 days
9	7	12 days	Brain virus heated 19 hours between 60 and 63 deg. C. and stored 2½ months	1½ c.c. 12 days after end of treatment	28% 14½ days	100% 13 2/7 days

REPORT OF CASES RECEIVING PASTEUR ANTIRABIC TREATMENT DURING 1908

D. W. Poor, M.D.

Treated at Laboratory.

	Diagnosis of Rabies in the Animal Certain	Diagnosis Probable.	Diagnosis Uncertain.	Animal Not Rabid.
Bitten on head	9		1	3
Bitten elsewhere	92	6	32	12
Contact of wounds with saliva of animals	11	1		3
Treatment Sent in City.				
Bitten on head	1			
Bitten elsewhere	14	6	5	1
Contact cases	20	2		
Treatment Sent out of City.				
Bitten on head	45	6	6	2
Bitten elsewhere	327	32	57	2
Contact cases	50			
	569	53	101	23
Total, 746				

CASES TREATED, BY MONTHS

January	45	August	62
February	38	September	65
March	80	October	67
April	61	November	47
May	67	December	54
June	82		
July	78		746

Residents, 200.
Non-residents, 546.

In addition a considerable number of persons took the treatment for a short time, pending the diagnosis in the dog. These cases are not included in the above report.

RECORD OF CASES WHICH DIED IN SPITE OF TREATMENT

	Age.	Location of Bite, Etc.	Period of Treatment.	Onset of Rabies.
Mrs. D.	42	Three penetrating wounds on bare arm, Aug. 30, A.M. Treated by doctor ½ hour later with carbolic acid, followed by alcohol and at laboratory, 3 days later with HNO_3.	Sept. 2 to 22 (both dates inclusive)	Pain in the arm, Sept. 23. Difficulty in swallowing water Oct. 1. Death a few days later.
G.R.	34	Five wounds on fingers, four penetrating and one lacerated. Cauterized with $AgNO_3$ Dec. 12.	Dec. 26 to Jan. 16	March 4, unable to drink. Died March 6.
J.W.	20	Two wounds on right side of nose and two on upper lip. March 2, cauterized immediately with carbolic acid.	March 8 to April 3 Patient skipped six days of treatment, four days at one time and two at another.	Died May 3
E.P.	5	Bitten on right ear, incised wound ⅛ inch deep, Jan. 13. Cauterized one hour later with $AgNO_3$.	Feb. 13 to Mar. 9	First symptoms of rabies, Mar. 9. Died Mar. 12.
Mr. F.	77	One penetrating wound on back of hand of moderate severity, and another fair sized wound on hand. Cauterized two hours later with HNO_3.	Apr. 14 to Mar. 3	First symptoms of rabies, July 14. Died July 20.

Three of the patients died of rabies after the period of observation. This gives a mortality of 4-10 of 1 per cent. in the total number treated in which the biting animal was certainly or probably rabid.

THE POWER OF CERTAIN DRUGS TO INHIBIT RABIC INFECTION

D. W. Poor, M.D.

The success obtained in the treatment of certain forms of trypanosome infection and also in that of syphilis by the use of atoxyl and mercury suggested the experimental trial of these drugs in the infection of rabies.

In the Zeitschrift für Hygiene, Vol. LIX, 1908, Heymann reports his experiments with atoxyl in attempting to prolong or prevent the onset of rabies. Rabbits were used, and the animals were first inoculated with rabies virus and then for several days treated with atoxyl, some subcutaneously, others intravenously, and still others by mouth. The action of atoxyl on rabies virus was also tested in vitro. His conclusions were that no effect was produced by this agent, either in the animal or in vitro, when given in doses that were non-toxic for the rabbits.

Since it was the combined use of atoxyl and mercury that had been successful in trypanosomiasis, we tried the effects of these agents together. In addition we studied the effect of chinosol and mercuric iodide, antiseptics theoretically serviceable for constitutional use.

In the experiments of Benjamin Moore, reported in the Biochemical Journal, 1907, rats were infected with trypanosomes, then given ½ c.c. of a 5 per cent. solution of atoxyl and later from 1.5 to 2.5 c.c. of a 1-1000 solution of mercuric chloride in divided doses.

The drugs were tested in the following strengths: Atoxyl, 5 per cent. solution; chinosol, ½ per cent. solution; mercuric iodide, 1-5000 solution; bichloride of mercury, 1-1000 solution.

Preliminary experiments showed that the non-toxic doses of these drugs for guinea pigs were:

Atoxyl, ½ c.c.
Chinosol, 4 c.c.
Mercuric iodide, 1 c.c.
Bichloride of mercury, ½ c.c.

TEST I

December 5th, 28 guinea pigs were given 1 c.c. of an emulsion of street virus in the leg.

Atoxyl and Mercuric Chloride

(a) On December 7th, seven of the pigs received ½ c.c. each of a 5 per cent. solution of atoxyl, and on December 9th, ½ c.c. of 1/1000 solution of $HgCl_2$.

Pig. No. 1, died December 10th, not of rabies.
Pig No. 2, died January 1st, rabies.
Pig No. 3, died December 11th, not rabies.
Pig No. 4, died January 2d, rabies.
Pig No. 5, remained well.
Pig No. 6, developed rabies January 16th.
Pig No. 7, developed rabies December 30th.

Mercuric Chrolide

(b) On the same date seven of the pigs received ½ c.c. each of 1/1000 HgCl solution On December 9th this was repeated.

Pig No. 1, died of rabies January 28th.
Pig No. 2, remained well.
Pig No. 3, remained well.
Pig No. 4, remained well.
Pig No. 5, developed rabies December 28th.
Pig No. 6, developed rabies January 8th.
Pig No. 7, developed rabies December 31st.

Controls

Pig No. 1, developed rabies January 4th.
Pig No. 2, developed rabies December 31st.
Pig No. 3, developed rabies December 29th.
Pig No. 4, developed rabies December 23d.
Pig No. 5, developed rabies December 31st.
Pig No. 6, died of rabies January 11th.
Pig No. 7, remained well.

Chinosol

(a) December 28th, 22 pigs were infected in the leg with 1 c.c. of emulsion of street virus. Of this number eight were inoculated subcutaneously with a 1/200 solution of chinosol as follows: December 31st, 1 c.c.; January 2d, 1 c.c.; January 4th, 1 c.c.

Pig No. 1, remained well.
Pig No. 2, remained well.
Pig No. 3, died of rabies January 12th.
Pig No. 4, developed rabies January 15th.
Pig No. 5, died of rabies January 27th.
Pig No. 6, developed rabies January 31st.
Pig No. 7, remained well.
Pig. No. 8, remained well.

Chinosol, Locally

(b) A second series of seven pigs received the chinosol solution locally in the region of the inoculation as follows: December 31st, 1 c.c.; January 2d, 1 c.c.

Pig No. 1, remained well.
Pig No. 2, remained well.
Pig No. 3, remained well.
Pig No. 4, remained well.
Pig No. 5, developed rabies January 20th.
Pig No. 6, died of rabies January 11th.
Pig No. 7, died of rabies January 16th.

Controls

Pig No. 1, remained well.
Pig No. 2, remained well.
Pig No. 3, remained well.
Pig No. 4 remained well.
Pig No. 5, developed rabies January 17th.
Pig No. 6, died of rabies January 20th.
Pig No. 7, died of rabies January 18th.

Iodide of Mercury

(c) Fourteen pigs inoculated in leg with 1 c.c. of street virus emulsions on February 6th. On February 7th seven pigs received 1 c.c. of a 1/5000 solution of mercuric iodide. On February 8th, 1 c.c. was given in site of inoculation, and on February 11th another injection of 1 c.c. was given subcutaneously.

Record

Pig No. 1, developed rabies February 2d.
Pig No. 2, developed rabies February 26th.
Pig No. 3, developed rabies March 15th.
Pig No. 4, developed rabies February 29th.
Pig No. 5, developed rabies February 19th.
Pig No. 6, developed rabies February 25th.
Pig No. 7, remained well.

Controls

Pig No. 1, developed rabies February 20th.
Pig No. 2, developed rabies February 20th.
Pig No. 3, developed rabies February 18th.
Pig No. 4, developed rabies March 2d.
Pig No. 5, remained well.
Pig No. 6, remained well.
Pig No. 7, remained well.

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THE DISTRIBUTION OF BACTERIA IN BOTTLED MILK AND ITS APPLICATION TO INFANT FEEDING*

MIDDLE MILK MIXTURES

By Alfred F. Hess, M.D., New York

So many experiments have been made and so much has been written upon the bacteriology of milk and the methods of obtaining pure milk, that it did not occur to me to inquire whether the question of the distribution of bacteria in milk was definitely understood. Some months ago, however, in the course of an investigation of tuberculous contamination of milk, I had occasion to centrifuge small amounts of milk and to make smears from the surface of the cream which formed in a firm layer at the top of the tube. Although I had realized that the cream of the milk contains relatively many bacteria, I was impressed with the fact that these smears, on staining, showed an immense number of various organisms, including numerous chains of streptococci. This led me to inquire more closely into the distribution of the bacteria in our bottled milk, where the cream has risen by the natural force of gravity. I set myself the task of finding out where the bacteria in an ordinary bottle of milk may be found; not only whether, and to how great an extent, they are to be found at the top or at the bottom, but whether the divers layers, if we divide the cream arbitrarily in strata, contain relatively constant quantities.

The only method applicable to this end seemed to be the removal of definite amounts from the bottle, for the purpose of making separate bacterial estimations. With this object in view, I obtained numerous bottles of different brands of milk and pipetted off successive layers of cream or skim-milk. The technic of counting was that in use at this laboratory. Although a pipette was employed in most instances to remove the milk, in two series a 1-ounce dipper was used, and in two others simple pouring was employed.

The tables will best explain the results obtained. A glance at the section A of Table I shows at once that the cream contains by far the

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* Read before the New York Academy of Medicine, Section on Pediatrics, April 9, 1908.

TABLE I

BACTERIA PER C.C. OF VARIOUS LAYERS OF BOTTLED MILK

A	(1st 50 c.c.)	(2d 50 c.c.)	(3d 50 c.c.)	(4th 50 c.c.)	(5th 50 c.c.)	(Lowest 50 c.c.)	
1	130,000	101,000	48,000	18,000	3,000	7,000	
2	48,000	34,000	27,000	14,000	1,000	4,000	
	(1st 100 c.c.)	(2d 100 c.c.)					
3	38,000	6,000					
	(1st 100 c.c.)	(Remainder)					
4	36,000	5,000					
	(Upper ½ cream)	(Lower ½ cream)	(Remainder				
5	116,000	17,000	1,000				
B	(1st oz.)	(2d oz.)	(3d oz.)	(4th oz.)	(5th oz.)	(6th oz.)	
1	108,000	70,000	58,000	41,000			
2	105,000	68,000	20,000	8,000	11,000	10,000	Poured
3	124,000	89,000	108,000	58,000	10,000	2,000	Dipped
4	243,000	184,000					
5	1,540,000	1,226,000	160,000				
C	(1st oz.)	(2d 2 oz.)	3d 2 oz.)	(Remainder.)			
1	235,000	96,000	76,000	40,000			
2	115,000	36,000	7,000	6,000			Poured
3	102,000	57,000	17,000				Dipped

greatest number of bacteria, and that the skim-milk, even in the lowest portions of the bottle, is relatively free. This has been brought out by Freeman and others. Heretofore, however, the cream has been regarded as a homogeneous unit, containing numerous bacteria in uniform suspension. Closer inspection of the table reveals that this is not the case; that in every instance the bacterial contamination is greatest in the uppermost cream, becoming always less and less as we approach the lean milk. This is equally true even in the milk which has been poured. The first ounce contains more bacteria than the second ounce, the second more than the third, and so on. The first two ounces, however, form a nidus for the greatest number of organisms. Section C of Table I demonstrates this point, but as it is essential to the practical application which I shall later suggest, I have framed a second table (Table II), which comprises counts of the first two ounces, the second

TABLE II

1st 2 oz.	2d 2 oz.	Remainder.
93,500	49,000	
86,500	14,000	
235,000	96,000	40,000
115,000	36,000	6,000
102,000	57,000	
1,383,000		160,000

two ounces, and, in some instances, of the remainder of the bottle. I would like to call attention to the last count of the series, where the first two ounces of the cream contained 1,383,000 bacteria, and the remainder of the bottle only 160,000 per c.c. In this instance, by removing the first two ounces, we freed the milk of about eight millions of bacteria. This principle applies not only to milk of high bacterial count, but also to milk coming well within the range of certified milk, such as number 2 in group C of the first table, in which the average count of the bottle was less than 15,000 per c.c. Here the first two ounces contained 115,000 germs per c.c., so that when we include this portion in our formulæ we, by this means, contaminate the remainder of our milk. Or, if we add the next two ounces, using the upper four ounces, as some advise, we even then make use of cream containing 75,000 bacteria to the c.c.

I was interested to ascertain whether tubercle bacilli were likewise carried upward toward the surface of the cream by the rising of the fat globules. For this purpose I prepared a homogeneous suspension of bovine bacilli, with which I infected "loose" milk. Two to five c.c. to the quart were used, and the milk was allowed to stand 24 hours. On the following day four guinea pigs were inoculated with specimens taken respectively from the first two ounces, the second two ounces, as well as from the skim-milk and lowest ounces in the bottle. The inoculations were made intraperitoneally, and one ounce injected, with the result that many pigs died of infection before tuberculosis could develop. I have prepared a table (Table III) of five such experiments, realizing that they are inconclusive, but believing that they show the preponderance of bacilli in the upper two ounces. In order to obtain

TABLE III

ANIMAL INOCULATION WITH TUBERCULOUS MILK

Experiment.	I.	II.	III.	IV.	V.
1st 2 oz. cream....	*	Tb.	Tb.	Tb.	Tb.
2d 2 oz. cream....	No Tb.	*	No Tb.	Tb.	2*
Skim-milk........	No Tb.	No Tb.	No Tb.	No Tb.	No Tb.
Lowest 2 oz......	*	No Tb.	No Tb.	Tb.	No Tb.

* Signifies that pig died of early septic infection.

further information upon this point, I inoculated milk with larger amounts of tubercle bacilli in suspension, but instead of using animal tests I made smears from the different levels. Table IV shows the results of two such tests. The smears demonstrated conclusively that tubercle bacilli are also carried upward by the fat globules. It was very striking to compare the number found at the very surface of the bottle with that from even the uppermost ounce of the cream.

TABLE IV

ANIMAL INOCULATION WITH TUBERCULOUS MILK

	Bottle A.	Bottle B.
Surface of cream....	Very large number of bacilli; some clumps.	Numerous bacilli; few clumps.
1st 2 oz............................	Few bacilli; 1 clump.	Few bacilli seen.
2d 2 oz.............................	No bacilli seen.	2 bacilli seen.
3d 2 oz.............................	1 bacillus seen.	1 clump seen.
Skim-milk...........................	No bacilli seen.	1 bacillus seen.
Lowest ½ oz. (dregs)................	Few bacilli seen.	1 bacillus seen.

The statistics which I have given for gravity cream do not apply for centrifugal cream. If we centrifuge milk rapidly and for a considerable period, and then make smears from the cream, from the skim-milk, and from the sediment, we find that although many bacteria are in the upper layer, the sediment also is rich in bacteria, and contains many more than previous to the separation. If we centrifuge less rapidly or for a shorter period of time and obtain a less dense layer of cream, the conditions approach more nearly those of gravity cream. In other words, the result depends upon the relation of the artificial centrifugal force to the natural centripetal force of gravity; if the former is very great, it will drive many of the bacteria to the sediment. I do not mean to infer by this that we are able to obtain a pure cream by means of the centrifuge, but merely that the fresh cream obtained by this method contains fewer bacteria than the gravity cream. I may also add that what I have said throughout concerning bacteria applies equally to leucocytes—that they are also carried upward by the fat and are usually found in great numbers at the surface of the cream.

Having dwelt at some length on the purely theoretical side of our subject, let us now turn to its practical application. At the present day every effort is being made to supply a milk with the lowest possible degree of contamination. The certified milk was a long stride in advance, but unhappily at the present time its cost greatly curtails its helpfulness, and the number of its beneficiaries is comparatively small. Any suggestion, therefore, which would help to procure a less contaminated milk for the masses is certainly worthy of consideration. Now that we realize that the upper two ounces of the bottle harbor the greatest number of bacteria, the removal of this portion naturally suggests itself. Of course by this procedure we lose butter fat as well as bacteria. A bottle of milk, which averages about 4.2 per cent. fat, I have found will contain but 3 per cent. when the upper two ounces have been removed. A 5 per cent. milk is reduced to a 3.25 to 3.5 per cent. milk. However, this may frequently be of advantage, as probably most of us will agree. As Holt says: "There are many healthy infants who cannot digest even 4 per cent. of fat at any time, and many more who during hot weather do much better when a reduction to 3 per cent. or 3.5 per cent. is made." It is hardly necessary for me to particularize the instances in which a 3 per cent. of milk is of value. I should like to draw attention, in passing, to the facility with which this "low-fat" milk is obtained, and to call attention to the fact that the method does not entail the necessity of pouring the milk into another vessel, and thus subjecting it to the danger of additional contamination; the cap needs merely to be reapplied to the bottle and the milk then shaken. A 3 per cent. milk procured in this manner contains fewer microorganisms than a similar milk obtained by the usual dilution of one-fourth water, and is less apt to contain tubercle bacilli, a danger by no means to be disregarded in the ordinary bottled milk. That its volume is less than the watered milk of equal richness may also be accounted an advantage, as surely nothing is to be gained by feeding an infant with large quantities of water. When we dilute a 4 per cent. milk one-fourth by adding 11 ounces of water to a quart, we obtain a 3 per cent. milk, but at the same time reduce the proteid to 2.6 per cent. if we consider the average proteid content of cow's milk to be 3.5 per cent. On the other hand, if we use the partially skimmed milk which I suggest, although the fat likewise equals 3 per cent., the proteids remain full strength, namely, 3.5 per cent. Considering the fact that the proteids are more frequently prescribed too low than too high in later infancy, this formula should prove useful.

As a basis for the current top-milk formulæ it is customary to use a 12 per cent, or a 10 per cent. milk, and a 7 per cent, milk; in other words, one formula in which the fat content is about four or three times that of the proteid, and another where it is only twice as great. In order to bring this method into conformity with these well-established principles, I have endeavored to duplicate these percentages, and have had various portions of bottled milk tested quantitatively for fat by the Babcock method, for which tests I wish to thank Mr. Congdon, of the Chemical Laboratory of the Department of Health, and Messrs. Harrison and Jeffers, of the Walker Gordon Laboratory. Many tests were made; the results are incorporated in Table V. The upper two ounces of the bottle contain on an average 24 per cent. of fat. This estimation and those that follow are based on 4 per cent. milk, which

TABLE V.

METHOD OF OBTAINING 12 PER CENT., 10 PER CENT., OR 7 PER CENT. MILK AFTER REMOVAL OF THE UPPER TWO OUNCES

	Fat.	Proteid.
Next 7 ounces	12 per cent.	3.2 per cent.
" 8 "	10 " "	3.3 " "
" 12 "	7 " "	3.5 " "

has been bottled about 24 hours; at any rate, for not less than 14 and not longer than 36 hours. As these two ounces are discarded, a slight variation in the fat percentage cannot result in serious error. It is true that if the milk has been bottled for 48 hours or more, this layer becomes considerably richer. After two ounces are removed, the next seven ounces contain 12 per cent. fat, 3.2 per cent. proteid; the next eight ounces 10 per cent fat and 3.3 per cent. proteid; the next 12 ounces contain 7 per cent. fat and 3.5 per cent. proteid. These figures are not absolute, as may be easily imagined; however, they compare very favorably with comparative tests made with top milk according to established methods.

I shall not weary you with an array of formulæ. The same formulæ may be prepared on this basis as are generally made use of in top milk preparations. I may add, however, that if one wishes to obtain a 4 per cent. milk with its normal quota of proteid, in other words, a full milk, one has only to add 3 ounces of the 10 per cent. cream to the 3 per cent. milk, which I have shown is so easy to prepare.

In closing, let me add that this method of preparing milk should prove particularly serviceable in the summer time, when we have so much to fear from bacterial contamination as well as from digestive disturbances due to a food too rich in fat.

My thanks are due to Dr. Park for suggestions freely given in the course of this work.

Summary

In bottled milk the bacteria are by far the most numerous in the upper layers of the cream, becoming gradually fewer in its lower portion.

The upper two ounces contain the greatest number of bacteria.

This is true of tubercle bacilli as well as of streptococci and other bacteria.

Therefore, instead of using the upper cream, as is now practiced, it is preferable to discard the upper two ounces.

The average bottle of such *partially skimmed milk* contains 3 per cent. fat and 3.5 per cent. proteid and is well adapted for infant feeding.

If we discard the upper two ounces we have: next seven ounces, a

12 per cent. milk; next eight ounces, a 10 per cent. milk; next 12 ounces, a 7 per cent. milk. With these figures as a basis the usual top-milk formulæ may be prepared.

AN INEXPENSIVE HOME-MADE MILK REFRIGERATOR

ALFRED F. HESS, M.D.

Individuals and communities are at present much interested in the question of pure milk for the infants of the poor. A more rigid supervision is gradually being enforced over all those who handle milk—the farmer, the dairyman, the wholesaler, and the retailer. Although these efforts can not be too highly commended, too little stress has been laid on the importance of the care of the milk in the home of the consumer. No matter how carefully the milk has been obtained and guarded up to the time it is retailed, even if it is pasteurized or certified, it will be rendered unfit for food after standing in a room at summer heat for a few hours. That this is a real danger is known to all who have tended babies in the tenement houses in summer. Most of the people in poor circumstances have no ice or an insufficient supply, so that the milk is kept at a temperature of from 50° to 70° F.

For some months I have been endeavoring to devise a simple and inexpensive means for keeping milk in summer—one that will be within the reach of the mother in the tenement house. After considerable experimenting, I can recommend the following box for this purpose:

An ordinary packing case was obtained; it had been made for bottled water, and measured on the inside 13 by 18 inches and was 11½ inches in depth. Sufficient sawdust was placed in this box to make a substantial layer on the bottom. On this was set a tin can, tall enough to hold a quart bottle of milk and 8 inches in diameter, and around this was placed a cylinder of tin a little larger in diameter than the can. The cylinder was then surrounded by sawdust. The lid of the can was, of course, left free. The ice box was completed by nailing about 50 layers of newspapers to the lid of the case (Figs. 1 and 2). The total cost of such an apparatus is the cost of the tin can, which may be 25 or 50 cents, according to the quality. The box and sawdust can be obtained free from a grocer.

To test the value of the box, a quart of milk was placed in the can and surrounded by 6 or 7 pounds of ice; that is to say, less than five cents worth. The room temperature was 81° F. The efficacy of the refrigerator was demonstrated by the fact that 24 hours later the temperature of the water in the can was 33° F., the milk in the bottle 37° F., and that even after 45 hours the temperature of the water had risen only to 50° F. and the milk to 52° F.

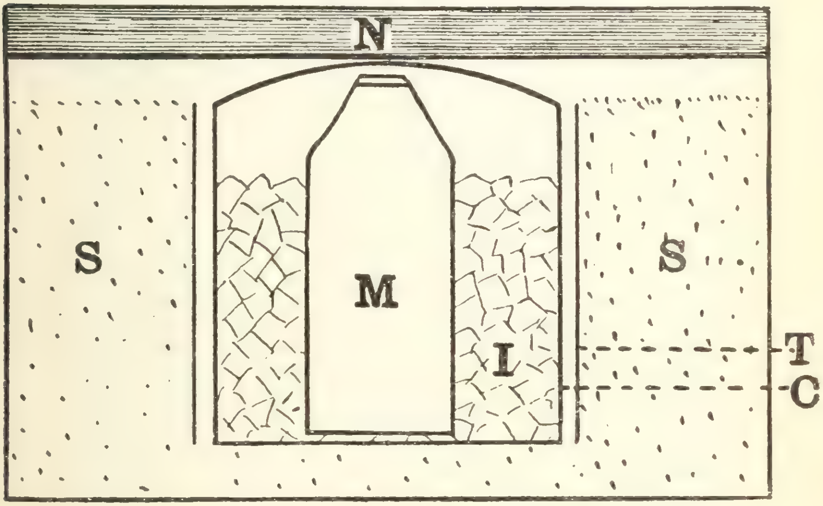

Fig. 1.—Vertical section of home-made milk refrigerator; S, sawdust, excelsior or other cheap non-conductor of heat; T, cylinder of oilcloth, tin or galvanized iron; C, can in which is placed the milk jar M, surrounded by broken ice, I; N, newspapers nailed to lid of case.

Numerous variations from this type of box were found to keep out the heat. A somewhat larger box was found more desirable. Excelsior may be substituted for sawdust. All that is necessary is that the can containing the ice be surrounded on all sides by a material which conducts heat poorly. Care should be taken that the can rests on sawdust and not directly on the wooden floor of the case. Should the case be rather shallow for the can, newspapers should be laid between the two. To prevent rusting a little soda may be placed in the can every day.

The apparatus described above will keep two quart bottles of milk, or four 8-ounce feeding bottles. The great majority of mothers in the tenements keep the day's supply of milk in a quart bottle and possess but two or three nursing bottles. As the ideal method is to have as many bottles as there are feedings in the course of the day, it was determined to make such minor modifications in the ice box as would allow of this procedure. To this end a tin can was obtained which was 8¾ inches in diameter and cost 30 cents. It was sufficiently large to admit a wire bottle holder costing 45 cents and containing eight bottles. A case 18 inches square was employed to hold it. The ice was cracked into smaller pieces than before, 6 or 7 pounds being used, and the wire holder with its bottles (previously cooled in running water) was then set on the surface of the ice, or rather gently pressed down into the ice. Within one hour the temperature of the milk fell from 67° to 55° F. and continued to fall. After 24 hours it was at 39° F. and the water at 38° F.

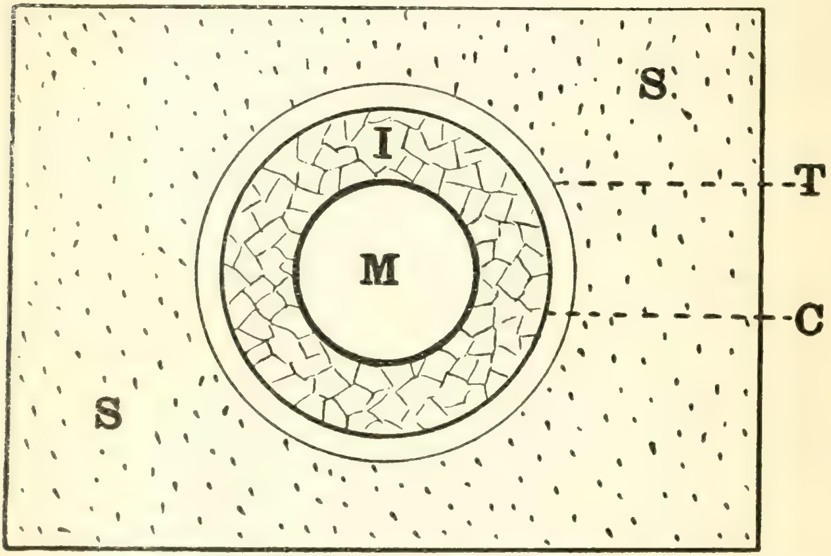

Fig. 2.—Horizontal section of home-made milk refrigerator; M, milk container; I, broken ice; C, can for holding ice; T, tin, oilcloth or galvanized iron cylinder to prevent sawdust, S, from falling into space when can is removed for purpose of emptying water.

The bacterial content of the milk was 7,000 bacteria to the c.c. when it was obtained. After 24 hours the milk in the refrigerator had risen to 42,000 to the c.c. A sample of the same milk left at a temperature of 73° F. showed 12,360,000 bacteria to the c.c.

Will mothers take the trouble to improvise ice boxes of this description? From an experience with mothers who consult the dispensaries I can say that they are anxious to do all in their power to protect their babies. Many would be quick to profit from the lesson if they saw a model of the ice box, were told how cheaply it can be constructed, how it will economize ice, and, finally, how its employment will aid in saving the baby from an attack of the much-dreaded summer complaint. The cost of such ice boxes can be considerably reduced if they are made in large quantities, so that with private and municipal co-operation they could be supplied for much less than the above figures.

Refrigerators of this design, 1½ feet square by 14 inches deep, have been distributed among the children's dispensaries, dairy kitchens, nurses' settlements and kindred organizations in New York City, where they are being demonstrated to mothers. It is hoped that this article may encourage other communities to similar action in aid of the poor babies.

A HANDBAG REFRIGERATOR FOR THE UNCONTAMINATED CARRYING OF MILK SAMPLES

Chas. B. Fitzpatrick, M.D.

Milk samples are frequently obtained in small glass bottles holding about 30 c.c. provided with a metal screw top containing a wafer of cork as a filling. These samples are often placed in ordinary handbags, with an inside tin or zinc lining, and kept cold by being directly surrounded by ice. The ice and ice-water often completely immerse the bottles, so that they are often found floating in this ice and water mixture. An examination of many of these samples showed that the bottles were not sufficiently water tight to prevent contamination of the milk samples. A simple way to avoid this source of contamination is as follows: (1) Take two round tin cans 5 inches in diameter and three inches in height (sufficiently large to hold 12 of these sample bottles), which are absolutely water-tight. (2) Put a false wooden bot-

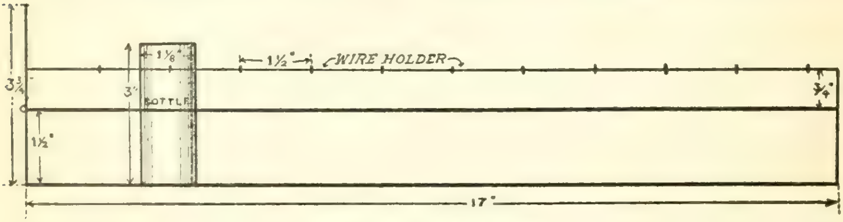

FIG. I. SIDE VIEW OF TRAY

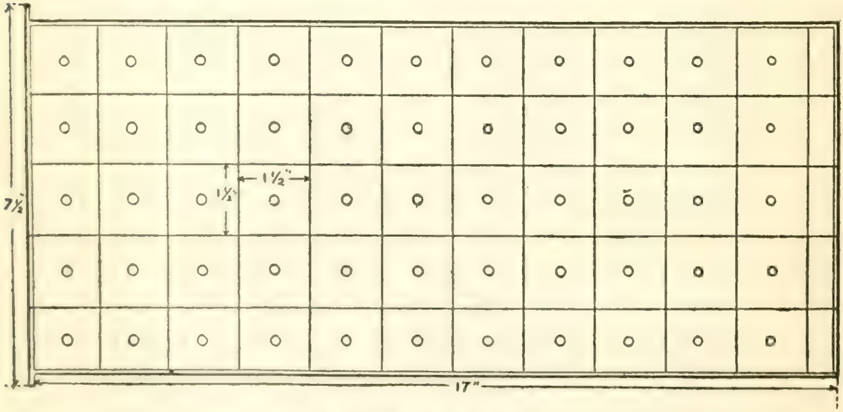

FIG. II. PLAN OF TRAY

tom in the bag by means of two rods running horizontally from one end to the other of the top of this bottom which reaches the middle of the bag, supporting the two boxes and separating them from the bottom half of the bag, containing the ice. The tin cans are placed immediately above the ice and supported by the two rods. This plan avoids all contamination and the temperature is often as low as 34½° F.

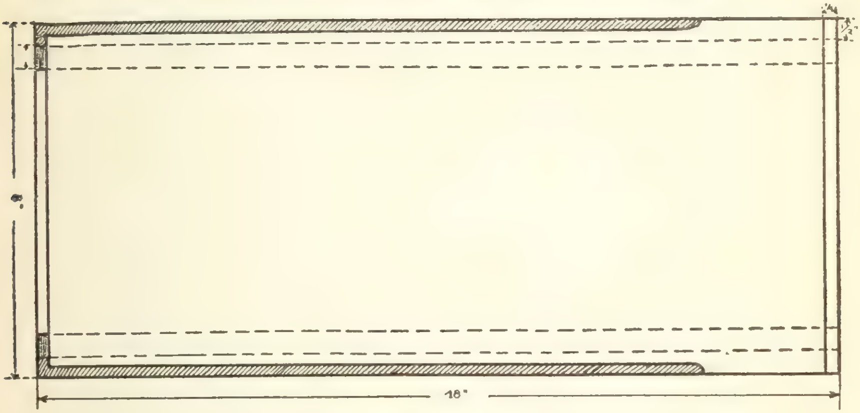

FIG. III. PLAN OF BAG

Another more elaborate way to avoid this contamination is to take an ordinary handbag and cut an opening in one end of it, so as to admit a tin drawer, approximately the length and width of the bag, abbreviated just enough to admit the drawer and to permit it to slide

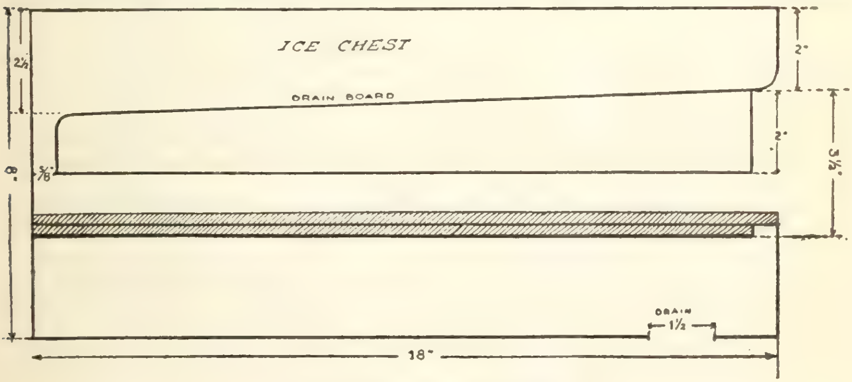

FIG. IV. SIDE VIEW OF BAG.

freely in and out. This drawer accommodates about 50 sample bottles and is completely separated from the upper half of the bag by a shelf of tin which contains the ice. This shelf is attached at the ends and sides to the inside tin container. The long side of this roof or shelf is bent down and extends so as to cover the bottle room and hangs down at the sides to below the middle of the bottles. This overhang carries the water of the melted ice below the bottles into the tin bottom of the bag, which is 1 inch lower than the drawer and contains a screw cap outlet for the water. The bottles are thus kept dry and away from the drip. A space of 1 inch is also allowed at the side between the row of bottles and the side overhang. One end of this shelf connects directly with the inside tin container. The bottom of the drawer contains fine perforations. The temperature in this bag, when ice is used, averages about 36° F. (Fig. I to VI incl.).

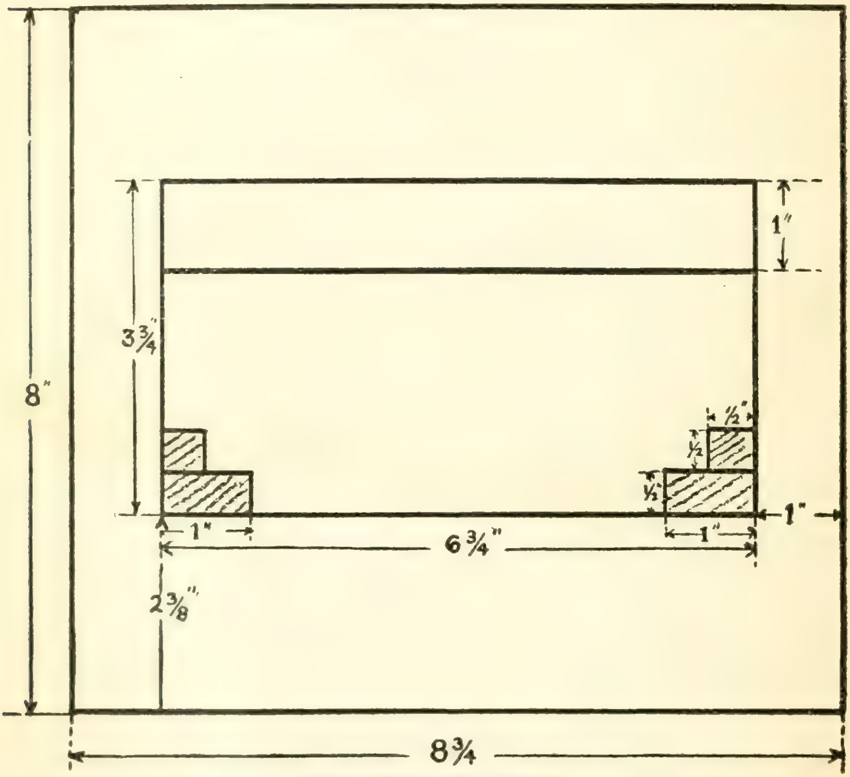

FIG. V. FRONT VIEW OF BAG

Various modifications may be made. In order to see how cheaply an efficient bag could be improvised, the two tin cans were taken and one was filled with ice while the other contained the samples. The one containing the ice was placed directly upon the top of the one holding the samples. A felt bottom was attached to this and another piece of felt was wrapped around it, thus providing sides. A felt cover was put on this and a piece of wire wound twice around the sides, the bottom and top were likewise held in place by two bands of wire placed at right angles, and a wire handle improvised. This made an efficient refrigerator that looked like an ordinary pail, and which could be made out of any pliable material that was a good non-conductor of heat.

This two separate containers idea may be also applied to the bag by simply making the shelf which divides the bag in two connect directly to all the sides, thus making two distinct non-communicating compartments. The drawer fits in as before described.

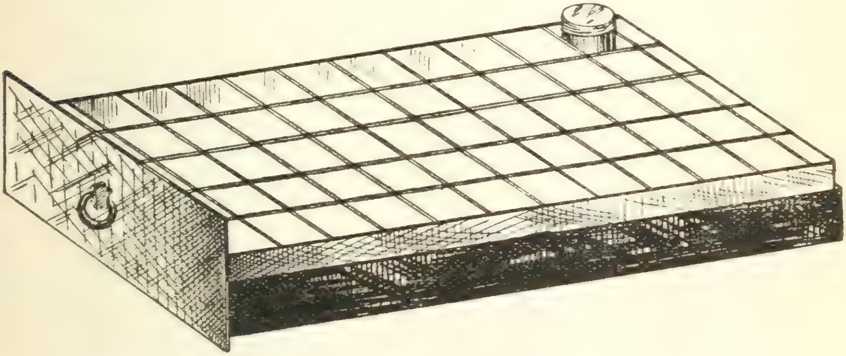

FIG. VI. SKETCH OF TRAY

A further improvement readily suggests itself, namely, to make a bag of one-half (or less) the length of the bag (Fig. I to VI incl.), designed to carry 25 (or less) bottles. The trays, each holding about 12 bottles, could also be arranged, so as to be detachable (together with the bottom of the bag), thus enabling one to have as small a bag as possible to carry for each occasion. I desire to thank Mr. Russell Raynor, Chief of the Divisions of Inspections, for his kindness in helping me to have this work finished. The ready co-operation of Milk Inspector Walters and Ass't Chemist Alexander is also acknowledged.

THE PRESENCE OF TUBERCLE BACILLI IN THE CIRCULATING BLOOD

BY

CHARLES KRUMWIEDE, JR.

That tubercle bacilli could be demonstrated in the circulating blood or in the blood obtained at autopsy, in a small number of cases has long been an accepted fact.[1] Recently Rosenberger[2] has examined the blood in 125 cases of tuberculosis and reported positive results in all. These cases embraced every degree of severity from slight infection to the acute miliary type. If tubercle bacilli are regularly present in the blood, and can be demonstrated with such certainty, Rosenberger's communication would be an important advance in methods of diagnosis. For this reason we have tried a small number of cases of frank pulmonary tuberculosis, most of them well advanced with numerous tubercle bacilli in the sputum.

The technique employed was that suggested by Rosenberger. The test tubes, pipettes and slides were all new, except in the first two cases, where stock pipettes were used.

Four cases and two controls were on the service of Dr. Nathaniel B. Potter at the French Hospital; five cases were from the Riverside Hospital, making a total of nine cases.

Besides the examination of slides, some of the sediment was spread over the surface of glycerin egg and the remainder injected into guinea pigs.

The microscopical examination was negative in all cases except one. In this case there were acid fast clumps, resembling tubercle bacilli, but not typical. They could be found in only two slides. Other slides from the sediment were completely negative. Blood was again taken from this case. Both samples were completely negative. Stock pipettes* were used in the first sample, new pipettes in the second and

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[1] Bacteriologie des Blutes bei Infectionskrankheiten. P. Canon (Fischer-Jena, 1905), p. 135.

[2] American Journal of Medical Sciences, February, 1909.

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* These pipettes, however, had never been used on tuberculous material.

third. This was the result in examining three slides from a case, each slide being examined one-half hour. Subsequently these slides were re-examined by Dr. G. B. Foster spending much more time on the individual slides, in some instances three hours, with the following results: One control case, clinically secondary syphilis with no suspicion of tuberculosis showed three clumps of eight to thirty bacilli. The duplicate slides from the case showing enormous numbers were found negative. In four of the cases of pulmonary tuberculosis which had been negative, one showed two small clumps and a few isolated bacilli, two showed a few isolated bacilli and one a few intracellular acid fast bodies. These isolated rods had been observed in the first examination but were thought not to resemble tubercle bacilli sufficiently to call the slide positive.

The attempts at cultivating tubercle bacilli from the sediment were negative in all cases.

As to the inoculations into guinea pigs, in two cases the pigs died acutely. The pig receiving the sediment which apparently gave a positive microscopic examination died in 21 days and was negative. The second sample from this case proved negative in pigs; the third died acutely. The pigs of the remaining six cases were completely negative.

After the above results we feel that the finding of isolated bacilli in blood smears of supposed tubercular patients would be a precarious method of diagnosis. The finding of clumps of bacilli raises the question, why, if they were so numerous as in one case, were not subsequent examinations also positive and why were culture and guinea pig inoculations negative? Further, the same acid fast bacilli were found in a case which had not the slightest suspicion of tuberculous infection. The argument that in the first case the bacilli were dead and only discharged into the blood at the time the examination was positive and that in the second case there was an undiscovered tuberculous lesion is slightly incredible.

We conclude that acid fast bacilli may be found, but that these findings are valueless in diagnosis. In view of the discordant results we feel that contamination from water or air is the most likely source of these acid fast bacilli, though we have no proof of this.

NOTES ON THE EXPERIMENTAL PRODUCTION OF FAGET'S DIAGNOSTIC REACTION OF YELLOW FEVER

By Charles B. Fitzpatrick, M.D.

Faget, from his studies of the New Orleans (1870) and the Memphis (1873) epidemics, discovered that a falling pulse with a rising or horizontal temperature is a diagnostic reaction, pathognomonic of yellow fever. His conclusions are widely followed in the South, and this reaction is considered by many physicians to be more or less pathognomonic of this disease.[1]

Hence, I have been led to briefly outline some results obtained by injecting the toxic contents of bacteria isolated from cases of yellow fever. The culture which furnished most of the reactions was isolated by me from the liver of a case of yellow fever and named B. coli icteroides.[2] The other culture was one of the B. icteroides, sent to me by Sanarelli; both of these cultures belong to the colon group. The B. coli icteroides was first tested on dogs. It was prepared for this purpose by being incubated for 24 hours in bouillon at 37° C., then for 24 hours at 40° C., and then left for 24 hours between 45° and 50° C., and finally was exposed for five minutes at 55° C. One dose of 15 cubic centimeters was injected subcutaneously into a healthy dog weighing 45 pounds. When the reaction had completely disappeared the same dog was inoculated with a fatal dose of the *living* culture of the B. coli icteroides. The dog recovered after a severe and protracted reaction. A control dog was inoculated at the same time with an equal amount of this living culture and it died in about 24 hours. This procedure was repeated with the b. icteroides and the same results were obtained. Another culture of the b. coli icteroides, slightly different from the one already employed, was similarly investigated and gave like results. These three cultures were then mixed together in equal parts, treated as the single culture and gave the same results. Finally, a sterile fluid was prepared of these three cultures, which had been grown for four days, then killed by exposure and heat and further disintegrated.

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[1] J. C. Faget, Yellow Fever, pp. 1-49, J. B. Balliere & Sons, Paris.

[2] Fitzpatrick, N. Y. Medical Record, Jan. 29, 1898, and June 1, 1899.

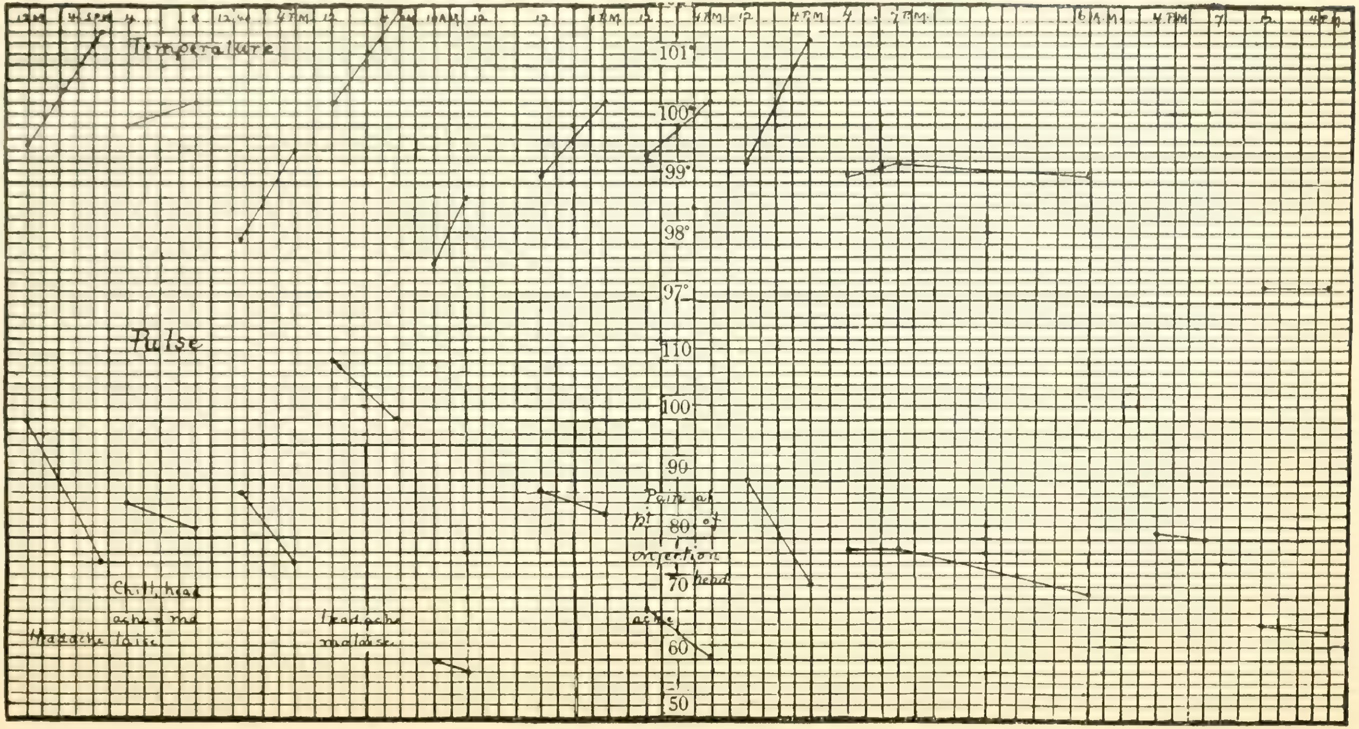
The use of this preparation in humans has given some interesting results. Eleven of the persons who were injected subcutaneously with from one-half of a c.c. to 5 c.c. reacted with the characteristic falling pulse and a rising or horizontal temperature, which is known as Faget's reaction of yellow fever. Chill, headache, malaise and pain at the point of inoculation were also noted.

CHART OF ELEVEN FAGET'S DIAGNOSTIC REACTIONS OF YELLOW FEVER

(i.e. Falling Pulse with Rising or Horizontal Temperature)

Experimentally produced in man by Subcutaneous Injections of Toxins obtained from B. coli commune (B. coli icteroides and B. icteroides)

1*(1 c.c.) 2*(5 c.c.) 3†(½ c.c.) 4†(2 c.c.) 5 (1 c.c.) 6 (1 c.c.) 7 102°(2 c.c.) 8 (2½ c.c.) 9 (2 c.c.) 10 (1½ c.c.) 11 (2 c.c.)



*Reactions 1 and 2 belong to one case.
†Reactions 3 and 4 also belong to one case.
The other reactions are each from separate cases.

Each No. (1 to 11) on this line indicates a separate reaction. The amount of subcutaneous injection is within the ( ) immediately following the number.

EXPERIMENTAL DATA RELATING TO HAEMOLYTIC SERA

BY

L. W. FAMULENER, M.D., and ALICE G. MANN

GENERAL OUTLINE

A. NATURAL IMMUNE BODIES IN NORMAL SERA
  I. Action of Normal Sera from Different Species upon Alien Blood Cells.
  II. Action of Normal Sera from Different Individuals of the Same Species upon Alien Blood Cells.
  III. Action of Natural Immune Body Influenced by an Alien Complement.
  IV. Action of Natural Immune Serum upon Blood Cells of Different Individuals of the Same Species.

B. COMPLEMENTING SERA
  I. Comparative Complementing Values of Rabbit, Sheep and Goat Sera with a Specific Immune Serum.
  II. Comparative Complementing Values of Guinea-pig Serum and Rabbit Serum with a Specific Immune Serum.
  III. Comparative Complementing Values of Guinea-pig Serum and Rabbit Serum when Acting upon Homologous Specific Immune Sera from Alien Species of Animals.
  IV. A Comparison of Sera from Normal Rabbits and Sera from "Snuffle" Rabbits as to Their Relative Complementing Action.

C. HAEMOLYSIS AS AFFECTED BY CONCENTRATION OF DIFFERENT COMPONENTS OF THE IMMUNE BODY—COMPLEMENT COMPLEX
  I. Constant Amount of Complement, Constant Volume of Fluid, Variable Number of Blood Cells and Decreasing Amounts of Immune Body.
  II. Constant Amount of Complement, Variable Volume of Fluid, Constant Number (approx.) of Blood Cells and Decreasing Amounts of Immune Body.

CONCLUDING REMARKS.

INTRODUCTORY REMARKS

Perhaps no group of experimental studies has had greater influence upon the general problem of immunity than that involving haemolytic sera and their reactions. These studies have not only embraced the immune body complement complex, but have been extended to include definite haemolytic toxins of bacterial origin and their specific antibodies. In many respects these bacterial toxins and antitoxins are analogous to those which hold such an important place in medicine. But their interactions can be studied with greater ease and probably with a greater degree of accuracy since the test tube method of investigation is applicable. By such a method the investigator has a means of controlling various factors and conditions of experimentation at his will, and is able to read the results with mathematical precision. It is quite obvious that a means of study is afforded which approaches the ideal—a condition far from attainable in purely animal experimentation.

The study of haemolytic sera has thrown much light upon the nature of cytolytic substances in the body fluids. Moreover the subject has been found to possess a practical application in other fields. Noteworthy in this connection may be mentioned the tests for blood stains (Deutsch, and the Neisser-Sachs) in medico-legal cases; the Bordet-Gengou reaction (fixation of complement), for the recognition of a large class of specific antibodies; the Wassermann test and its various modifications in the diagnosis of syphilis; investigations in connection with the cancer problem; the study and differentiation of micro-organisms into sub-groups; the examination for the presence of normal haemolysins prior to transfusions, etc.

Although an extensive literature has developed in connection with this subject, many of the basic data are fragmentary, and so widely dispersed throughout the whole that they are hardly accessible. It is often necessary for the individual worker to do much preliminary work in order to establish data before undertaking a definite problem involving these reactions. Since the writers have had to cover this ground, preliminary to certain haemolytic studies, it was deemed worth while to collect some of their data and make them accessible for future reference. In some cases additional work has been done for the purpose of further completeness, although no attempt has been made to cover all conditions. The subject matter to follow will include certain tests which may throw light upon particular experimental conditions, or may act as a check to prevent errors in technic. The protocol tables will be given intact in some cases; in other instances various steps will be tabulated in order to indicate the development of the method used; some results will be shown by diagrams, while others will be represented graphically by curves. Explanatory notes will be given in connection with each series of tests.

This work will be confined to those reactions in which haemolytic sera enter, and will include immune body and complement relations. It is taken for granted that laboratory workers in this particular line have an understanding of the fundamental theories pertaining to the interaction between cells, immune body and complement. Such information may be derived from recent text-books on bacteriology, pathology, or special works on the subject of immunity.

A. NATURAL IMMUNE BODIES IN NORMAL SERA

I—Action of Normal Sera from Different Species upon Alien Blood Cells

It is a well-known fact that the blood serum of one species of animal may dissolve the blood cells of an animal of another species; that is, possesses a haemolytic action for those particular blood cells. While on the other hand, the serum of like species does not dissolve the homologous blood cells, except under certain abnormal conditions. But the serum of the alien species does not always possess this haemolytic action; in fact, it may be quite inert.

Since the discovery of natural immune bodies (haemolytic), and complement in fresh normal serum, an explanation is given for such phenomena. Haemolysis only takes place after the particular blood cells have become sensitized by the absorption of immune body, and then through this combination become subject to the action of complement. In the absence of either specific immune body or complement haemolysis does not take place.

The appended table (No. 1), taken from our tests, offers an excellent illustration of how, in some cases, the presence of natural immune bodies acts against alien blood cells. It also shows how such bodies in a single serum may act upon the blood cells of several species of animals. Dog serum, for example, haemolysed the blood sells of horse, goat, sheep, rabbit and guinea pig.

TABLE I

NATURAL HAEMOLYSIS IN NORMAL SERUM

Key:—

Comp. H. = Complete solution (haemolysis) of blood cells.
+++ = Almost complete haemolysis of blood cells.
++ = Partial haemolysis of blood cells.
+ = Definite coloration of supernatant fluid.
Sl. + = Lesser coloration of supernatant fluid.
t. = Only a tint in supernatant fluid.
Sl. t. = Slight tint in supernatant fluid.
o = No coloration in the supernatant fluid—water white.

Dog Serum.	*5% Susp. of Blood Cells.				
	Horse.	Goat.	Sheep.	Rabbit.	†G. P.
1.00 c.c.	+++	+++	+++	Comp. H	Comp. H
0.80 "	+++	+++	+++	+++	"
0.60 "	+++	++	+++	+++	"
0.50 "	+++	++	++	+++	"
0.40 "	++	+	++	+++	"
0.30 "	++	+	++	++	"
0.25 "	++	+	+	+	"
0.20 "	++	t.	+	+	+++
0.15 "	+	o	t.	t.	+++
0.10 "	+	o	o	o	++

Horse Serum.	5% Susp. of Blood Cells.				
	Goat.	Sheep.	Rabbit.	G. P.	Dog.
1.00 c.c.	o	o	+	t.	o
0.80 "	o	o	+	sl.t.	o
0.60 "	o	o	+	o	o
0.50 "	o	o	t.	o	o
0.40 "	o	o	t.	o	o
0.30 "	o	o	sl.t.	o	o
0.25 "	o	o	"	o	o
0.20 "	o	o	o	o	o
0.15 "	o	o	o	o	o
0.10 "	o	o	o	o	o

Goat Serum.	5% Susp. of Blood Cells.				
	Sheep.	Rabbit.	G. P.	Dog.	Horse.
1.00 c.c.	o	Comp. H	Comp. H	o	+++
0.80 "	o	"	"	o	++
0.60 "	o	"	"	o	++
0.50 "	o	"	"	o	+
0.40 "	o	"	+++	o	t.
0.30 "	o	+++	++	o	sl.t.
0.25 "	o	++	+	o	"
0.20 "	o	+	t.	o	o
0.15 "	o	t.	o	o	o
0.10 "	o	o	o	o	o

Sheep Serum.	5% Susp. of Blood Cells.				
	Horse.	Goat.	Dog.	Rabbit.	G. P.
1.00 c.c.	++	o	o	+++	Comp. H
0.80 "	++	o	o	+++	"
0.60 "	+	o	o	+++	"
0.50 "	+	o	o	++	"
0.40 "	t.	o	o	++	"
0.30 "	sl.t.	o	o	+	"
0.25 "	o	o	o	sl.t.	"
0.20 "	o	o	o	o	+++
0.15 "	o	o	o	o	++
0.10 "	o	o	o	o	+

Rabbit Serum.	5% Susp. of Blood Cells.				
	Goat.	Sheep.	Horse.	G. P.	Dog.
1.00 c.c.	t.	t.	Comp. H	Comp. H	o
0.80 "	t.	o	+++	"	o
0.60 "	sl.t.	o	+++	"	o
0.50 "	o	o	+++	"	o
0.40 "	o	o	+++	+++	o
0.30 "	o	o	+++	+++	o
0.25 "	o	o	+++	++	o
0.20 "	o	o	+++	+	o
0.15 "	o	o	++	t.	o
0.10 "	o	o	++	sl.t.	o

Guinea Pig Serum.	5% Susp. of Blood Cells.				
	Sheep.	Rabbit.	Goat.	Dog.	Horse.
1.00 c.c.	t.	sl.+	sl.+	t.	sl.+
0.80 "	t.	"	t.	t.	t.
0.60 "	sl.t.	t.	o	t.	o
0.50 "	o	o	o	o	o
0.40 "	o	o	o	o	o
0.30 "	o	o	o	o	o
0.25 "	o	o	o	o	o
0.20 "	o	o	o	o	o
0.15 "	o	o	o	o	o
0.10 "	o	o	o	o	o

* Final mixture of serum, salt sol. (0.9%), and blood cells, gave 3.0 c.c. of 5% suspension of corpuscles.

† Guinea Pig.

The tests recorded in this table were made by selecting six of the more available laboratory animals and drawing sufficient blood from either the jugular vein or the carotid artery into a sterile flask containing a spiral of medium sized wire. The blood was defibrinated at once by vigorously shaking the flask, the fibrin for the most part collecting about the wire. The whole blood was poured off into large centifuge tubes, and the cells separated from the serum by centrifuging. Then the supernatant serum was removed by pipette, put in bottles, and placed into the ice box until ready for use. To the corpuscles remaining in the tube several times their volume of 0.9 per cent. NaCl was added, and the whole well shaken, again centrifuged, the supernatant fluid from the blood cells removed, then the washing procedure repeated several times, until the cells were practically free from any appreciable amount of serum. After the last washing, and removal of supernatant fluid, the well packed corpuscles were drawn off by pipette and measured. Just before the blood cells were to be used in the experiment, a 7½ per cent. suspension was prepared in 0.9 per cent. NaCl solution.

Suitable test tubes were placed in holders in series of ten each, and into these was pipetted the fresh serum (containing complement and natural immune bodies) in decreasing amounts as indicated in columns under "——— Serum" in table. Sufficient 0.9 per cent NaCl solution was added to bring the volume of fluid up to 1.0 c.c. in each tube, then 2.0 c.c. of the blood cell suspension (7½ per cent. corpuscles) were added to each and the whole well shaken at once. The resulting mixtures in test tubes were equivalent to 3.0 c.c. of 5 per cent. blood cell suspension, with decreasing amounts of complement and immune body. The tubes were placed in the incubator at 37° C. for one hour, then removed, thoroughly shaken again, and placed in the ice box. After 12 to 14 hours the corpuscles which were not haemolysed settled to the bottom of the tube. When haemolysis had taken place the supernatant fluid showed different degrees of red coloration or "laking." Readings were made of the haemolysis by recording the amount of "laking" roughly indicated by the marks given with the table (see key). The result of the tests may be briefly summarized as follows:

1. Dog serum; shows a marked hæmolytic action upon the blood cells of the horse, goat, sheep, rabbit and guinea pig.

2. Horse serum; no haemolytic action upon the blood cells of goat, sheep and dog; a slight action upon guinea pig corpuscles, while rabbit corpuscles were moderately haemolysed.

3. Goat serum; marked haemolytic action upon rabbit, horse and guinea pig corpuscles; no action upon blood cells of sheep or dog.

4. Sheep serum; marked haemolytic action upon guinea pig blood cells; somewhat less upon those of rabbit, and moderate action upon horse corpuscles. No action upon goat or dog corpuscles.

5. Rabbit serum; marked action upon horse and guinea pig corpuscles; very slight action upon goat and sheep blood cells. No action upon dog blood cells.

6. Guinea pig serum; only a slight haemolytic action upon the blood cells of sheep, rabbit, goat, dog and horse.

It is noteworthy that in the above tests in no case did the alien serum haemolyse the dog corpuscles, while the dog's serum haemolysed all the alien blood cells. Since only one dog was used, it is possible that these results were peculiar in this case. Its serum was opalescent, containing a fat-like substance.

In these special tests the immune bodies were activated only by the amount of complement present in the serum under examination; that is, the fresh serum was used without the addition of any other complementing serum. As is evident, the introduction of an alien serum for complement would have greatly complicated or even invalidated certain of the tests. But as will be demonstrated later, the amount of complement present in a fresh serum may be quite insufficient to fully activate all of the natural immune body present. In such case the complete haemolytic power is not brought out in such tests.

Certain of the tests forming parts of the table were repeated with similar results each time. However, in other cases only one test was made, notably in the case of the dog serum, and dog blood cells in relation to the other sera and bloods. Exceptional cases have been reported where the sera from individuals of like species varied greatly in action upon the blood cells of an individual of an alien species—the serum of one animal might produce marked haemolysis, while that from a second would be without action. The same variation has been found in the resistance of blood cells, from different members of the same species, to the lytic action of a particular serum of another species. We shall have occasion to take up this point more fully later. These facts will account for the discordant reports which occur in the literature pertaining to natural haemolysins.

In practical work where a specific haemolytic serum is being used, it is important to select for complement a serum which has no natural immune body for the specific blood cells used in the experiment. Also, for most cases, it is better to select blood cells for immunizing purposes which are not normally haemolysed by the fresh serum of the animal to be treated. The futility of the opposite procedure becomes evident in cases, where, for example, transmitted immunity from mother to offspring is under investigation. In this connection the table submitted may be of value as a reference guide, but, as pointed out above, the sera of individuals vary in their haemolytic properties; likewise blood cells are more or less variable in resistance, so preliminary control tests should always be made on animals selected for haemolytic studies.

II—Action of Normal Sera from Different Individuals of the Same Species upon Alien Blood Cells

In order to compare the relative values of natural immune bodies in sera from a given species of animals, five normal goats were bled, the blood defibrinated, and the serum removed. The fresh sera thus prepared were added, in decreasing amounts, to series of tubes containing a given amount of horse corpuscles from a normal animal. The final mixture of goat serum, blood suspension and physiological salt solution gave 3 c.c. volume, containing 5 per cent. suspension of blood cells. This general technic was followed throughout the entire series of experiments. The mixture in the tubes was well shaken, placed in incubator at 37° C. for one hour, then removed and again shaken, placed in ice box until the whole cells settled to the bottom of the tubes, leaving a supernatant transparent fluid above. The first column in Table II indicates the amount of serum used; the following columns indicate the different goats and the results of the test. In examining the table it will be observed that the serum of no two animals showed equal amounts of immune body, lytic to the red blood cells of the horse. Under goat "A" the striking fact is brought out that certain animals may be an exception to the general rule that goat serum haemolyses horse blood cells. Apparently all graduations of lytic power may exist. The serum of goat "D" showed only slight haemolytic action, while that of "B," "E" and "C" showed an increasing strength in the respective order given.

It must be remembered that in this experiment we are not dealing with natural immune body alone, but also with complement. The fresh serum while containing both of the active haemolytic elements may not contain sufficient complement to activate all of the immune body which is present. Further, the complement of that same animal which is furnishing the immune body is not necessarily the most active one (for that particular immune body) which may be had. Therefore, in this experiment it must be understood that these factors may, and at times certainly do enter, thus altering the real values. But, the test serves to demonstrate that the amount of a particular natural immune body in the serum of adult individuals of the same species is not constant, even when the animals have been kept under the same conditions, etc.

TABLE II

VARIABLE HAEMOLYTIC ACTION OF SERA FROM NORMAL GOATS UPON HORSE BLOOD CELLS

Amount Goat Serum.	Goat "A."	Goat "B."	Goat "C."	Goat "D."	Goat "E."
1.00 c.c.	0	++	+++	sl.+	+++
0.80 "	0	+	+++	"	+++
0.50 "	0	+	++	t.	++
0.30 "	0	sl.+	++	sl.t.	++
0.20 "	0	t.	++	0	t.
0.10 "	0	0	++	0	sl.t.
0.05 "	0	0	sl.+	0	0
0.02 "	0	0	0	0	0

III—Action of Natural Immune Bodies as Influenced by an Alien Complement

As pointed out above, it is questionable if a fresh serum containing both natural immune body and complement ever contains sufficient of the latter to activate the entire content of immune body. This point was tested by drawing blood from a normal goat, defibrinating and removing the serum after centrifuging. The serum was divided into two portions; one portion was placed in the ice box until ready for use; the other was "inactivated" by heating at 56° C. for 30 minutes in a water bath. The heating destroys the complementing action of the serum without appreciably affecting the immune body. A normal guinea pig was placed under slight anaesthesia (ether), then bled to death from the carotids. The blood was defibrinated at once, and the serum was removed from the blood cells after separation by centrifuging. This serum was used in amount of 0.10 c.c. per tube as additional complement in certain series of the experiment. In control tests it was found that fivefold this amount of guinea pig serum exercised no haemolytic action upon the blood cells of the horse which supplied the corpuscles for the test. By consulting Table III the amounts of goat serum used in each series will be found recorded in the left hand column. In column "I" only fresh or "active" goat serum was added (in decreasing amounts) to series of tubes to which were added 2 c.c. of 7½ per cent. suspension of horse blood, then sufficient physiological salt solution to bring total volume to 3 c.c., or equivalent to a 5 per cent. blood suspension; column "II." the same, with the exception of 0.10 c.c. guinea pig serum for additional complement; column "III," the heated or "inactivated" goat serum was used, quite the same as in series "I"; while in column "IV" the "inactivated" serum was added as in series "III," with the addition of 0.10 c.c. guinea pig serum from complement.

The results as indicated in the table (III) show that the natural immune body of goat serum, in this case at least, when activated by its own complement, possessed marked haemolytic action (I). But when additional complement was added (II), the haemolytic action was increased, that is, in the first series more immune body was present than necessary for the amount of native complement present, so that the full haemolytic power was not brought forth in that case. In the series "III" where no "active" complement was present, naturally no haemolysis took place. However, in the last series, "IV," when 0.10 c.c. guinea pig serum was added for complementing action to the "inactivated" goat serum, the haemolytic power was practically restored, about equal to that which originally existed in the fresh goat serum.

We see, therefore, that haemolytic sera may depend very much upon the kind and amount of complementing serum present for their maximum action. These points will be more fully developed later.

TABLE III

INFLUENCE OF ADDITIONAL ALIEN COMPLEMENT UPON NATURAL IMMUNE BODY IN GOAT SERUM, ACTING UPON HORSE BLOOD CELLS

Amount Goat Serum	I. Active Goat Serum.	II. Active Goat Serum + 0.1 c.c. Comp.	III. Inactive Goat Serum.	IV. Inact. Goat Serum + 0.1 c.c. Comp.
0.20 c.c.	+	++	o	+
0.15 "	+	++	o	+
0.10 "	t.	++	o	t.
0.08 "	sl.t.	+	o	t.
0.06 "	"	+	o	sl.t.
0.05 "	v.sl.t.	t.	o	"
0.04 "	"	sl.t.	o	v.sl.t.
0.03 "	o	v.sl.t.	o	o
0.02 "	o	"	o	o
0.01 "	o	"	o	o

IV—Action of Natural Immune Serum Upon Blood Cells of Different Individuals of the Same Species

Previously it has been pointed out that different sera of the same species of animal possess variable haemolytic action upon the corpuscles of another animal. It might be of interest to know whether the corpuscles of certain individuals possess relatively greater or less resistance to haemolysis than other members of the same species. For this purpose bleedings were made from six different horses which had been immuned against diphtheria toxin—the only horses available. Obviously the use of such animals' blood for this test is open to objection, still on the whole all had undergone like treatment. If the injections of toxins altered the blood cells to any appreciable extent, this change would be as a "constant," running throughout all of the animals. Apparently any marked individual difference in blood cells would show in the tests. After the blood was drawn, defibrinated and serum removed, the blood cells were vigorously washed in 0.9 per cent. NaCl solution to remove all traces of free serum or antitoxin. The technic of the tests was carried out as outlined previously. The immune body was derived from blood serum of normal goat—a natural immune body. Table IV (a) will make the experiment clear, while the diagram IV(b), showing a comparative colorimetric scheme which practically represents the table in skeleton form. The schematic curve, however, gives a better comparison of the lytic variability existing between the different individuals' corpuscles than is brought out in the table. It was obtained by making a colorimetric comparison throughout the different series of horse bloods used. For example, the third tube in the first series (horse No. 323), which contained 0.5 c.c. of the goat serum, was taken as an arbitrary "standard color" tube; it showed a definite degree of coloration due to the haemolysis which had taken place in it. This "standard" was matched with those in the second series (horse No. 338), and it was found that the second tube (containing 0.8 c.c. goat serum) in that series corresponded with the standard color tube; it was so marked in the diagram. When compared with the tube in the third series (horse No. 343) it was found that the color of the third tube (0.5 c.c. goat serum) was considerably less than the "standard," while the second tube (0.8 c.c. goat serum) was somewhat greater; but the standard color closely approached it, therefore it was estimated that a slightly less amount of serum (perhaps 0.7 c.c.) would have given an equivalent degree of haemolysis. So the point was located accordingly. In the same way the tubes of the remaining series were compared with the "standard" and corresponding values were estimated and plotted in the diagram. Parallel colorimetric curves with the same general contour were also derived by taking as "standard color" tubes those with greater or less degree of haemolysis (color) than the one used in the particular case which was plotted. Several such curves in an experiment act as controls upon each other, and upon the test as a whole. For experiments where more exact colorimetric curves are to be derived, the amounts of the active serum or substances used should be much closer together than in this case, since rather wide gaps exist between the amounts of serum used in the consecutive tubes. After considerable experience the readings and estimations can be made with a high degree of accuracy.

In a rough way this is the general method which is in common use in comparative haemolytic work when constructing a colorimetric curve. But in some cases, instead of selecting one of the tubes from

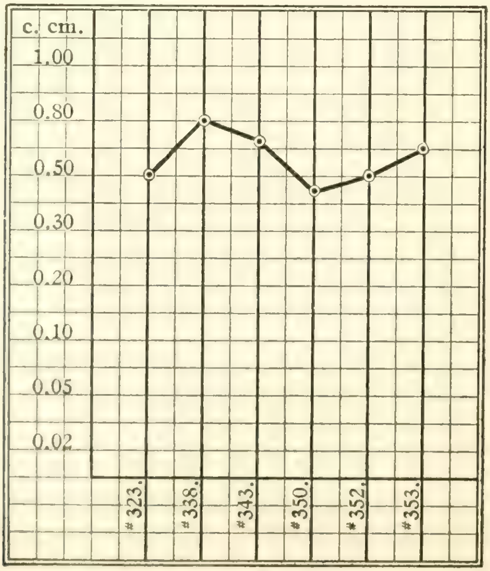
TABLE IV (a)

INDIVIDUAL VARIATION OF BLOOD CELL RESISTANCE TO HAEMOLYSIS

Amount Goat Serum.	Blood Cells from Different Horses.					
	No 323.	No. 338.	No. 343.	No. 350.	No. 352.	No. 353.
1.00 c.c.	++	+	++	++	++	++
0.80	++	+	+	++	+	+
0.50 "	+	+	sl. +	+	sl. +	sl. +
0.30 "	sl. +	sl. +	sl. +	sl. +	t.	t.
0.20 "	t.	sl. +	t.	sl. t.	sl. t.	sl. t.
0.10 "	t.	t.	t.	0	0	0
0.05 "	sl. t.	sl. t.	sl. t.	0	0	0
0.02 "	0	0	sl. t.	0	0	0

DIAGRAM IV (b)

COMPARATIVE RESULTS FROM TABLE IV (a)



the series as a "standard," as in this case, a special colorimetric series is made by "laking" a given volume of red blood cells in distilled water. Then giving that solution a numerical value of 100, further dilutions are made from the original by adding more distilled water so that tubes may be prepared, showing 90 per cent., 85 per cent., etc. This allows relative percentage readings to be made of the haemolysis in a series of tubes in a test, provided the number of blood cells have been sufficiently limited; haemolysis of large quantities of blood corpuscles give a very deep coloration, which is difficult to read unless diluted out. This diagrammatic method will be used in connection with other tables, but must not be confused with certain curves which will appear and be explained later. A variation exists in lytic susceptibility of the different blood cells when acted upon by the same haemolytic serum. It is possible that the serum from another goat might have acted differently if it had been tested in parallel on the same blood cells. But this was not tried at the time.

Plainly this brings out the importance of using only the blood corpuscles of one animal in any particular haemolytic test. That is, the blood of one individual should not be used in one portion of a test embracing a series of haemolytic sera, and the blood of a second individual be used in another portion even if the percentages are equal, since this introduces a serious error in the results. Of course where one animal will not furnish sufficient blood for a test, the blood of a second or third can be drawn, and all well mixed, after washing. Then of the mixture ample suspension can be made up for the entire test just before use. Blood suspensions should always be made from freshly washed corpuscles shortly before adding to the tubes of the prepared haemolytic series, then well shaken at once to insure rapid and uniform mixing.

B. COMPLEMENTING SERA

I—Comparative Complementing Values of Rabbit, Sheep and Goat Sera with a Specific Immune Serum

While it is a well-known fact that all fresh blood serum contains complement, it is not commonly appreciated that the activity of sera from different species varies greatly one from the other in this respect.

The same holds true, to less extent, for sera of individuals of the same species. Some workers apparently hold to the view that the proper complementing serum should be derived from a normal animal either of the same species as the animal which has been immunized (against blood cells) or from the species which furnished the red blood cells for the immunization. For efficiency of complement, serum from either source is often inferior to that taken from other species. The experiment as recorded in Table V very well demonstrates this proposition.

A normal goat had been immunized against blood cells of sheep by repeated subcutaneous injections until its serum became strongly haemolytic to those cells. It may be recalled that normal goat serum contains little or no natural immune body against blood cells of sheep. Three series of test tubes had decreasing amounts of the immune serum added, as indicated in left hand column; the complement naturally present had become inactive by the ageing of the serum. To one series, 0.20 c.c. rabbit serum per tube was added; to the second series, the same amount of sheep serum; and to the third, an equal amount of goat serum. The serum for complement in each instance was taken from normal animals, and used while fresh. The technic employed was the same as in the previous tests; the blood suspension was made from well washed normal sheep blood cells. The results show that there is a

TABLE V

RELATIVE COMPLEMENTING VALUES OF SERUM FROM RABBIT, SHEEP AND GOAT

Immune Serum. (Goat).	Compl. Serum. (Rabbit) 0.20 c.c.	Compl. Serum. (Sheep) 0.20 c.c.	Compl. Serum. (Goat) 0.20 c.c.
0.20 c.c.	+ + +	sl. +	t.
0.15 "	+ + +	t.	sl.t.
0.10 "	+ + +	0	0
0.08 "	+ +	0	0
0.06 "	+ +	0	0
0.05 "	+ +	0	0
0.04 "	+ +	0	0
0.03 "	+	0	0
0.02 "	+	0	0
0.01 "	sl. +	0	0

marked difference in the complementing values of the respective sera for this particular immune body.

It was not the serum from the species which supplied the immune body (goat), but the serum from another species (rabbit), which showed the greatest complementing activity. The normal goat and sheep sera were equal in complement content, but far lower than rabbit serum. This shows the importance of a proper selection of normal serum for haemolytic work to be most efficient. Certain other factors also enter into the above experiment, but these will be discussed in connection with the following experiments.

II—Comparative Complementing Values of Guinea Pig Serum and Rabbit Serum with a Specific Immune Serum

In order to compare the sera of animals of the same species to each other, and this whole series to a second series of another species as to complementing values, the following test was undertaken: Three normal guinea pigs and the same number of rabbits were bled to death from carotids (ether anaesthesia), and the serum removed by clotting. Six series of tubes were prepared, as indicated in Table VIa, and to each

TABLE VI (a)

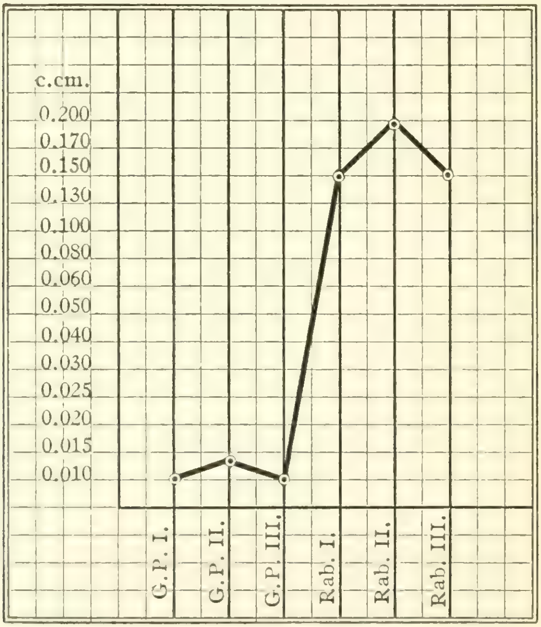
RELATIVE COMPLEMENTING VALUES OF A SERIES OF GUINEA PIG SERA AS COMPARED TO A SERIES OF RABBIT SERA

Amount of Compl. Serum.	G. P. I.	G. P. II.	G. P. III.	Rab. I.	Rab. II.	Rab. III.
0.20 c.c.	Comp. H.	Comp. H.	Comp. H.	+ +	+	+ +
0.17 “	“	“	“	+	sl. +	+
0.15 “	“	“	“	sl. +	sl. t.	sl. +
0.13 “	“	“	“	t.	o	t.
0.10 “	“	“	“	o	o	o
0.08 “	“	“	“	o	o	o
0.06 “	“	“	“	o	o	o
0.05 “	“	“	“	o	o	o
0.04 “	“	+ + +	“	o	o	o
0.03 “	“	+ +	+ + +	o	o	o
0.025 “	+ +	+ +	+ +	o	o	o
0.020 “	+ +	+	+ +	o	o	o
0.015 “	+	+	+	o	o	o
0.010 “	sl. +	t.	sl. +	o	o	o

tube was added 0.02 c.c. serum from a goat highly immunized against blood cells of sheep. Then complementing serum from the different animals was added in decreasing amounts, as shown in the column on the left. The series of tubes contain a constant amount of the same immune body, with variable amounts of the respective complement, as indicated at the head of each column. The regular technic was carried out as to blood suspensions, volume, incubation, etc.

DIAGRAM VI (b)

RESULTS OF TEST, FROM TABLE VI (b)



The result may be had by a glance at diagram VIb; it will be noted that in this particular case the series of guinea pig sera contains much more active complement than the series of rabbit sera. Moreover the guinea pig sera were about equal in complement content; the rabbit sera were more variable, one sample in particular was considerably weaker than the other two in complement stuff. Experience shows that for all practical purposes the guinea pig furnishes the most active complementing serum. Unfortunately, owing to the small size of the animal, the amount of serum is quite limited. In tests of large series of haemolytic sera, rabbit serum stands next in importance, since these animals furnish much more active serum than any of the large animals met with in our experience.

III—Comparative Complementing Values of Guinea Pig Serum and Rabbit Serum when Acting upon Homologous Specific Immune Sera from Alien Species of Animals

The question whether complement is a single, simple homogeneous substance found in fresh serum, or a heterogeneous substance showing a multiplicity of closely similar stuffs more or less specific in character, has long been open to debate. As yet the question has not been definitely decided to the satisfaction of all workers in this field.

Without entering this controversy in any way, we feel that it would be of interest to submit the following experiment, which might add something to the data on the subject.

TABLE VII

NORMAL SERUM FROM TWO DIFFERENT SPECIES AS COMPLEMENT TO IMMUNE SERUM FROM A THIRD SPECIES

Amount of Compl. Serum.	G. P. Complement.		Rabbit Complement.	
	*Immune Serum "A."	†Immune Serum "B."	Immune Serum "A."	Immune Serum "B."
0.50 c.c.	Comp.H.	Comp.H.	Comp.H.	Comp.H.
0.40 "	"	"	"	+++
0.30 "	"	"	"	++
0.25 "	+++	"	"	++
0.20 "	++	"	"	+
0.15 "	++	"	++	+
0.10 "	++	"	+	sl.+
0.08 "	+	"	sl.t.	0
0.06 "	+	"	"	0
0.05 "	+	"	0	0
0.04 ".......	sl.+	"	0	0
00.3 "	"	"	0	0
00.2 "	"	+++	0	0
0.015"	0	++	0	0
0.010"	0	+	0	0

*Immune serum from goat against blood cells of sheep.
†Immune serum from rabbit against blood cells of sheep.

A goat which had been immunized against red blood cells of sheep furnished a specific haemolytic serum, which will be indicated by "A." A rabbit was immunized in the same way against sheep blood cells, and gave a specific serum which will be designated by "B." A normal guinea pig and a normal rabbit, each had blood drawn, defibrinated and serum separated by centifuge. Each serum was used fresh as complement. Four series of tubes were prepared, and to each tube was added 0.05 c.c. immune serum—two series from serum "A" and the other two from serum "B." These series were subdivided into groups, each group having "A" and "B" series of immune serum.

In one group guinea pig serum was added in decreasing amounts to the series for complement, while in the other group rabbit serum was added in the same way in parallel. By consulting Table VII the scheme will be readily understood; the left column indicates the amount of complement used.

The results of the test show that the guinea pig complement was more active, in each case, in conjunction with the immune sera, than the rabbit complement. Also, that under its influence (?) immune serum "B" was more strongly haemolytic than immune serum "A." While on the other hand, the rabbit complement influenced (?) the immune body "A" to become more haemolytic than immune body "B," although not to the great extent as was the variation between the two series in the guinea pig complement group. These conflicting results would perhaps be explained by one school of workers as being due to

TABLE VIII

COMPARISON OF NORMAL RABBIT SERUM TO DISEASED RABBIT SERUM FOR COMPLEMENTING VALUES.

Amount of Compl. Serum.	Normal Rabbit "A."	Normal Rabbit "B."	Normal Rabbit "C."	"Snuffle" Rabbit I.	"Snuffle" Rabbit II.	"Snuffle" Rabbit III.
0.15 c.c.	++	Comp. H.	Comp. H.	Comp. H.	Comp. H.	+++
0.13 "	++	"	"	"	+++	++
0.10 "	+	++	++	++	++	++
0.08 "	+	sl. +	++	++	+	+
0.06 "	t.	o	sl. +	+	sl. t.	+
0.05 "	sl. t.	o	sl. t.	+	o	sl. +

"multiplicity of complements," in each of the normal sera, and that the specific elective action on their part in the two different immune sera gave the paradoxical results. On the other hand it might be urged that either serum, besides carrying immune bodies and complement, also carries other substances which might react primarily with the haemolytic elements, or among themselves in such a way as to secondarily affect the immune body complement complex. It is quite probable that the immune bodies themselves are not absolutely similar in character or structure. Many factors, still little understood, probably influence the haemolytic reactions. In submitting this test, we wish to point out in particular the importance of using only one specific immune serum and one particular complementing serum throughout any series of investigations involving the physico-chemical relations of these bodies.

IV—A Comparison of Sera from Normal Rabbits and Sera from "Snuffle" Rabbits as to Their Relative Complementing Action

Most serum laboratories have had more or less experience with a commonly known rabbit infection called "snuffles." As a general thing these diseased animals either die or undergo a long illness which makes them worthless for many experimental purposes. It was thought worth while to compare the sera from a series of these diseased animals, showing different degrees of acute infection, with the sera from healthy rabbits for value of the complement. Three normal rabbits were bled from the ear veins, while one "snuffle" rabbit was bled in the same way, and two others from carotids.

In each the serum was separated from the clot after the blood had stood in the ice box over night. Series of tubes were prepared according to the scheme given in Table VIII. To each tube was added 0.035 c.c. of specific immune serum of rabbit immunized against blood cells of sheep. Complementing serum from the different rabbits was added in amounts shown in first columns. Blood suspension, as in all previous tests, was added, and the routine technic was carried out. The result may be seen from the table.

It is quite interesting to note that the average values of the complementing serum from the "snuffles" rabbits was a little higher than that of the normal rabbit. For some purposes these diseased animals are of equal value for complement as the normal rabbit, and in our experience have been used to furnish complement for testing the haemolytic values of large series of samples of specific immune sera. The results were apparently accurate in every respect, as compared with sera from normal animals. Perhaps the use of such serum would not be so good for tests involving "fixation of complement" or others of the same character. These conclusions are only for this single infection. Other infections have not been tested by us.

C. HAEMOLYSIS AS AFFECTED BY CONCENTRATION OF DIFFERENT COMPONENTS OF THE IMMUNE BODY COMPLEMENT COMPLEX

I—Constant Amount of Complement, Constant Volume of Fluid, Different Concentrations of Blood Cells, and Decreasing Amounts of Immune Body

Certain investigators have carried out work upon the immune body complement complex from the physico-chemical standpoint. It has been shown that haemolytic action apparently follows certain recognized physico-chemical laws, and that the reactions may be expressed by mathematical formulæ. We shall not go into this side further than to call attention to certain combinations which have a practical bearing from the technical standpoint. Such conditions may arise during the course of determining the "immunity curve" in a series of sera derived from an animal immunized against blood cells. In this connection we shall point out the error arising when a perfectly homogeneous blood suspension is not used—that is, when a variable number of blood cells are present in equal volumes of fluid in the same series.

An experiment demonstrating the variations in haemolysis under such conditions was carried out as follows: Five series (reading from top to bottom of table) of test tubes were arranged, and to each series were added decreasing amounts of immune body (serum of rabbit immunized against sheep corpuscles), as indicated in Table IX (a). Each tube then received 0.2 c.c. fresh serum from normal rabbit for complement, and 0.9 per cent. salt solution enough to bring the volume of immune body and complement up to 1.0 c.c. in each tube. Finally different blood suspensions were prepared of such concentration that when 2 c.c. were added to the respective series of tubes, the resulting mixture (3 c.c. vol. in each tube) gave the percentage as indicated in the table, 1 per cent., 2 per cent., 3 per cent., etc.

The mixtures in the tubes were well shaken and then incubated at 37° C. for one hour, again shaken and placed in the ice box over night. Comparative colorimetric readings of a given degree of haemolysis were reckoned throughout the entire series, and designated by the character Δ.

TABLE IX (a)

HAEMOLYSIS AS INFLUENCED BY BLOOD CELL CONCENTRATION IN CONSTANT VOLUME OF FLUID

Amt. Immune Body	Blood suspension, Vol., 3 c. c.				
	1%	2%	3%	4%	5%
0.005 c.c.	—	—	—	—	⊤ Δ
0.004 "	—	—	⊤ Δ	⊤ Δ	⊥
0.003 "	—	—	Ш	⊥	—
0.0025 "	—	⊤ Δ	—	—	—
0.0020 "	—	Ш	—	—	—
0.0015 "	Δ	—	—	—	—
0.0010 "	—	—	—	—	—

By referring to table IX (a), it is evident that, by another arrangement, the test tubes would fall into series different from those just given. That is, reading from left to right on the horizontal line seven series of tubes would be formed. Each of the tubes in any given series (as, for example, the line headed by 0.005 c.c. immune body), would contain the same amount of immune body, the same amount of complementing serum, the same volume of fluid, but different numbers of blood cells (concentrations) as compared to others in the same series. A colorimetric curve could have been constructed on this basis

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Note.— Δ "standard color" tube selected from 1% series to make comparison for colorimetric curve.

⊤Δ⊥ "standard color" would fall about half way between the two tubes indicated.

⊤ΔШ "standard color" would fall very close to the tube connected by the three lines below.

by making a direct percentage reading of the amount of "laking" or haemolysis shown in each tube by comparing with a series of standard percentage color tubes, as explained under Section A, Division IV. Such a reading would show a decrease from greater to less haemolysis as we pass from left to right, or from a less to a greater blood cell concentration (1 per cent. to 5 per cent.). But similar results may be deduced from the colorimetric curve IXc (equal degree of haemolysis), which is given in connection with this discussion.

To return to the colorimetric readings shown in the table, we must add that such readings can be controlled in some cases by comparing "observed values" in each series with "estimated values" when the reaction follows a definite law which is expressible by means of a known mathematical formula. In this case the numerical value of △ in the experiment was determined by taking the amount of immune body indicated, at that point in each series where the haemolysis (color) corresponded with the original "*standard*" tube △. The sixth tube from the top in the "1 per cent. series" was used as a "*standard color*" tube. For the construction of a curve representing the results of the test, the reciprocal values were taken of the corresponding amounts of immune body (closely approximated in some cases), as indicated by △ in each column of the different series.

The table IXb, giving the "observed values" and the "reciprocal values," will make the method clear. In plotting the curve (curve IXc) only 1-10 of the reciprocal values have been taken, since that scale answers fully as well to bring out the relative values between

TABLE IX (b)

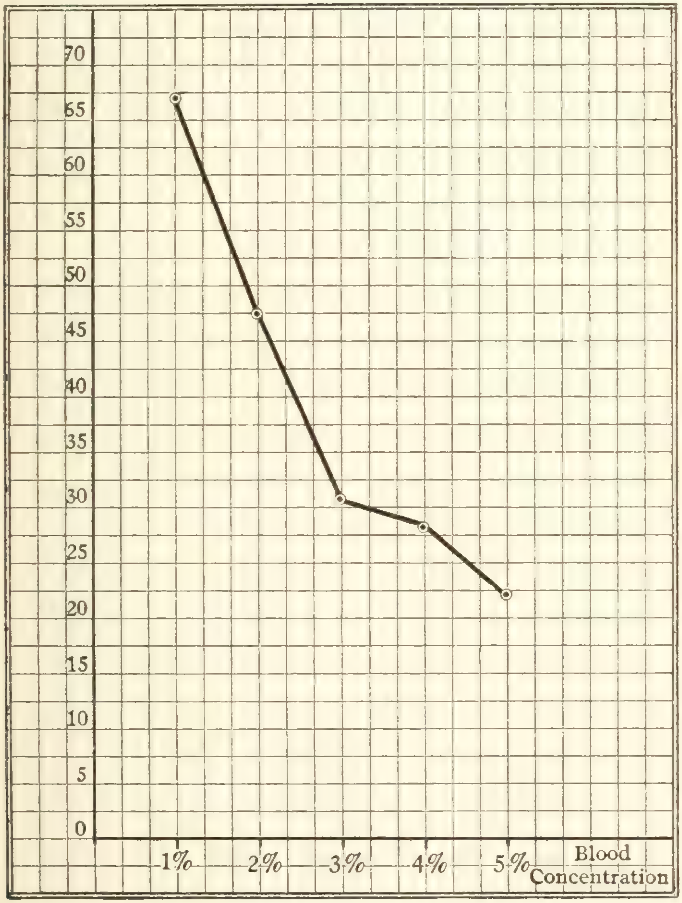
NUMERICAL VALUES FROM TABLE IX (a) FOR "COLOR CURVE"

Percentage Series.	Observed Values.	Reciprocal Values.
1%	0.0015	667
2%	0.0021	476
3%	0.0032	313
4%	0.0035	286
5%	0.0045	222

the series, the object of the test. The ordinates show the reciprocal values derived (IXb), while the abcissa indicates the different percentage of blood concentrations in the series. In a general way this

CURVE IX (c)

CURVE SHOWING THE INFLUENCE OF BLOOD CONCENTRATION UPON HAEMOLYSIS



experiment is parallel to the test ordinarily used to determine the haemolytic value of a specific immune body in any series of sera. But in this case our serum was used throughout with five different percentages of blood, instead of only one definite percentage of blood cells. The results of this test show that a marked variation in haemolysis does take place when all factors, excepting the number of blood corpuscles present are constant. A higher degree of haemolysis was produced in the lower percentages of blood dilutions, as shown upon plotted curve. Therefore, during any experiment or test of this character it is highly important that the blood mixture (blood cells in 0.9 per cent. NaCl solution) be kept well shaken during the pipetting off of the blood. The upper and lower layers of the blood mixture after 20 to 30 minutes may vary markedly as to concentration of corpuscles. Obviously this may be a means of pronounced error in the final results.

II—Constant Amount of Complement, Variable Volume of Fluid, Constant Number (Approximate) of Blood Cells and Decreasing Amounts of Immune Body

In connection with the previous experiment it was thought to be of interest to vary the concentration of the different elements of the immune body complements complex, with constant number (approximate) of blood cells. Five series of tubes were prepared, reading from top to bottom in Table Xa, and to each of these series were added decreasing amounts of a specific haemolytic serum (rabbit serum against sheep blood cells); each tube had an equal volume of complement, 0.10 c.c. fresh guinea pig serum.

From a concentrated suspension of blood of a definite strength, equal amounts were added to each tube throughout the entire series, giving approximately the same number of blood cells in each tube. But physiological salt solution was first added to each series, so that the first series contained sufficient fluid to give a 1 per cent. blood suspension of corpuscles in the total mixture; the second series received less salt solution, so that the final mixture was equivalent to a 2 per cent. blood suspension; and so on by lesser dilutions giving series of tubes with 3 per cent., 4 per cent. and 5 per cent. blood suspension. As apparent, the volume of fluid in the 1 per cent. series was five times greater than the volume in the 5 per cent. series. And the concentration of the haemolytic elements decreased as the volume of the fluid increased The technic of the experiment was quite the same as in previous tests. After the reaction between corpuscles, immune body and complement was completed, sufficient salt solution (0.9 per cent.) was added to the entire series of tubes to make the volume of fluid equal in each throughout. The mixture was well shaken, placed in the ice box to allow the

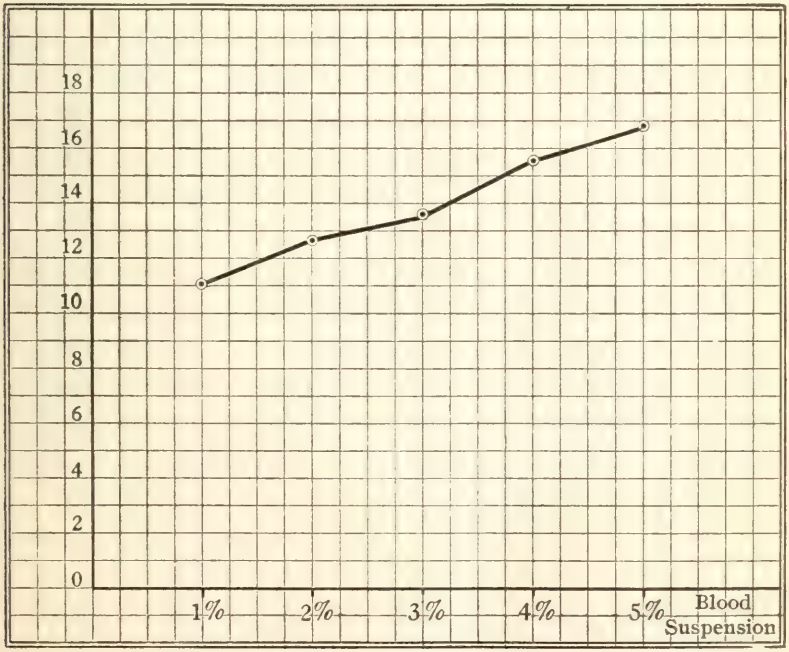
TABLE X(a)

INFLUENCE OF CONCENTRATION OF IMMUNE BODY, COMPLEMENT, AND BLOOD SUSPENSION UPON HAEMOLYSIS

Amt. of Immune Serum 1: 200	Constant number of blood cells				
	1%	2%	3%	4%	5%
0.10 c.c.	— ▲	—	—	—	—
0.08 "	—	— ▲	— ▲	— ▲	—
0.06 "	—	—	—	—	— ▲
0.05 "	—	—	—	—	—
0.04 "	—	—	—	—	—

CURVE X (b)

CURVE FROM TABLE X (a)



remaining intact corpuscles to settle out, after which the colorimetric readings were made.

A colorimetric comparison was run through the series, as shown in Table X (a), and from the reciprocal values of the numbers found a curve X (b) was plotted. It will be observed that haemolysis was greater in the series where the mixture of elements was more concentrated (5 per cent.) and diminish downward to the least concentrations (1 per cent.). This experiment might be read in another way by making a special arrangement of the tubes into different type of series. That is, make the reading of the table from left to right along the line which shows, as in the first case, 0.10 c.c. immune serum. In such an arrangement each tube of the series, as shown running from left to right in the table, would contain the same amount of immune body, a constant amount of complementing serum, a constant number of blood cells, but a variable amount of fluid (0.9 per cent. NaCl solution), giving different concentrations of all the elements in the haemolytic system.

This is practically parallel to the table in the section above. A curve could be gotten in the same way as was pointed out in that connection, so we shall refer to that section to save repetition.

When this experiment is compared with the preceding one, a striking contrast is found to exist. In the former case, the concentrations of the haemolytic components were equal in each series—only the number of corpuscles were variable in same volume of fluid. In this case the elements throughout varied in a regular order, as explained. Considered from the standpoint of blood concentrations, it is seen that in comparing the same concentration of both experiments, as the 5 per cent. series, in the first experiment the least haemolysis was induced, while in the second the greatest haemolysis took place. Among the factors which alter the results of haemolytic actions, concentration of the haemolytic elements also plays an important role. As demonstrated in this last test, it is always important to bring the volume of fluid in each test tube to a given volume, constant throughout the series, even when a constant amount of blood and serum is used.

CONCLUDING REMARKS

In submitting the above data, together with the methods used in developing the same, we trust that certain facts have been brought out which may prove to be of practical value to other workers in this field. While certain parts of the work are only a repetition of work done by other investigators, other materials have been brought forward which may throw light upon particular cases, and serve as a guide to prevent technical errors under certain conditions. Much of the elementary technic has been omitted since that material is available in many of the recent works which cover the subject of immunity.

We wish to acknowledge the assistance rendered by Dr. David Flynn and Miss Helen Hussey in connection with certain parts of the laboratory work.

REPORT OF THE BACTERIOLOGICAL EXAMINATION OF FECES FROM TYPHOID CONVALESCENTS, POST-TYPHOID AND NORMAL CASES FOR THE PRESENCE OF TYPHOID BACILLI

BY

W. CAREY NOBLE and JOSEPHINE S. PRATT

In the early spring of 1907 Dr. Goodwin, of this laboratory, isolated typhoid bacilli from the feces of Mary M., who was suspected by Dr. Soper of being a typhoid carrier. This case has been under our observation ever since, and during this time, the stools tested every week, with few exceptions, have continued to show many typhoid bacilli. (See Vol. III Collected Studies.[1])

The investigation of this typhoid carrier suggested to us first, the study of the persistence of typhoid bacilli in the stools of patients who are recovering from typhoid fever; second, the persistence of typhoid bacilli in the stools of patients who have passed the convalescent state, and may be classed as post-typhoid cases; and third, the study of stools from normal or non-typhoid cases for the possible presence of typhoid bacilli.

TYPHOID CONVALESCENTS AND POST-TYPHOID CASES

We began our investigation by the study of the persistence of typhoid bacilli in the stools of typhoid convalescents. These were obtained from 118 cases from the following institutions: Bellevue, St. Vincent's, Gouverneur, The City Hospital and Work House, Long Island State Hospital and the Smith Infirmary. To the doctors and nurses of these institutions we wish to express our thanks for aid and interest in the work.

The stools were collected in sterile bottles, care being taken that they should be uncontaminated by urine, and were brought to us as soon after collection as possible. A loopful of feces was then diluted in a sterile broth tube, and a loopful from the tube was streaked over a Conradi-Drigalski plate. Usually three or four plates were made from each specimen of feces. These plates, after incubation for 24 hours at 37°C., were examined for typhoid-like colonies. These colonies were fished into sterile nutrient broth and incubated for 24 hours at 37°C. They were then tested for agglutination with a high-grade anti-typhoid horse serum. The hanging drop method was used and a laboratory typhoid culture (Mt. S.) was used as a control. The cultures which agglutinated were then tested on the following media: broth, agar-agar, gelatin-slab, gelatin plate, litmus milk, glucose peptone water fermentation tubes, neutral red lactose peptone water fermentation tubes, potato and Dunham's peptone solution (a test for indol being made after ten days' incubation at 37°C.). The growth on the different media was compared with that of the laboratory culture of typhoid (Mt. S.) used as control.

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[1] Report on the Bacteriological Examination of a Typhoid Carrier. Collected Studies from the Research Laboratory, 1907, p. 193.

In planning the work, it was our hope to be able to make at least three examinations from each case, as we realized that a negative result from one stool was by no means indicative of the absence of typhoid bacilli in the feces. Unfortunately, however, we were not able to do this in many cases, for a report of a negative result of an examination was sometimes misinterpreted and a report of a positive result was sometimes disregarded and the patient was discharged from the hospital while still in a condition to spread the disease. In 50 of the 118 cases but one stool was obtained.

Of these 118 convalescent cases of typhoid, 15, or 12.7 per cent., retained the bacilli in the feces after the temperature had become normal. Five cases were discharged from the hospital, while their stools still showed enormous numbers of the bacilli; eight cases retained the bacilli from one to three weeks and two cases have been under observation for one and one-half years and are still positive.

The number of stools examined from the 15 cases in which typhoid bacilli were found is given on following page.

We found here, as in our previous work, that the proportion of typhoid organisms varies greatly. A positive stool showing only one or two typhoid colonies per plate would often be followed by one showing almost a pure culture of typhoid, or a negative stool would be followed by a positive one.

Case Number.	Number of Stools Examined.	Stools in Which Typhoid Bacilli Were Found.
1	12	1
2	9	1
3	6	1
4	4	1
5	4	2
6	7	2
7	24	18
8	34	6
9	3	1
10	1	1
11	1	1
12	1	1
13	1	1
14	65	53
15	67	54
Total 15 cases	239 Stools Examined	144 Positive Stools

As may be seen below, most of those who still harbored typhoid bacilli in their feces during convalescence did so for about one to three weeks only. Of the five who were discharged from the hospital while their stools still showed enormous numbers of typhoid bacilli we were able to trace only one.

Two tests of this patient's feces made three months later were negative for typhoid. Cases 14 and 15 have been under observation for one and one-half years and are still showing large numbers of typhoid in the stools.

	Sex.	Temperature Normal.	Result.	Remarks.
Case 1	F.	1st Test, 5 Days	—	Case was discharged from the Hospital.
		2nd " 7 "	+	
		3rd " 8 "	—	
		4th " 9 "	—	
		5th " 11 "	—	
		6th " 12 "	—	
		7th " 14 "	—	
		8th " 22 "	—	
		9th " 23 "	—	
		10th " 25 "	—	
		11th " 28 "	—	
		12th " 35 "	—	
Case 2	F.	1st Test, 4 Days	+	Case was discharged from the Hospital.
		2nd " 9 "	—	
		3rd " 10 "	—	
		4th " 11 "	—	
		5th " 12 "	—	
		6th " 13 "	—	
		7th " 16 "	—	
		8th " 17 "	—	
		9th " 26 "	—	
Case 3	F.	1st Test, 7 Days	+	Patient discharged from the Hospital.
		2nd " 17 "	—	
		3rd " 26 "	—	
		4th " 41 "	—	
		5th " 47 "	—	
		6th " 54 "	—	
Case 4	M.	1st Test, 7 Days	+	Patient discharged from the Hospital.
		2nd " 27 "	—	
		3rd " 34 "	—	
		4th " 41 "	—	
Case 5	M.	1st Test, 15 Days	+	Patient discharged from the Hospital.
		2nd " 16 "	+	
		3rd " 28 "	—	
		4th " 32 "	—	
Case 6	F.	1st Test, 17 Days	—	Patient (an insane woman) had a severe case of typhoid with relapse.
		2nd " 19 "	+	
		Relapse		
		3rd Test, 10 Days	+	
		4th " 17 "	—	
		5th " 24 "	—	
		6th " 31 "	—	
		7th " 38 "	—	

	Sex.	Temperature Normal.	Result.	Remarks.
Case 7	M.	1st Test Dec. 9, '07	+	Patient was still running a temperature when the first 15 tests were made. He was admitted to the Hospital Dec. 8, 1907.
		" 10	+	
		" 11	—	
		Dec. 12, 12, 13, 14, 15	+ + + + +	
		" 16	—	
		" 17, 18, 20, 20	+ + + +	
		" 21, 22	+ +	
		Dec. 23 T.N. 1 Day	—	
		" 24 2 Days	—	
		" 25 3 "	+	
		Jan. 1, '08 7 "	+	Patient was discharged from the Hospital.
		14 "	+	
		16 "	+	
		17 "	+	
		20 "	—	
		21 "	—	
Case 8	M.	Temp. not yet normal		
		1st Test	+	
		2nd "	—	
		2nd & 4th Tests	+ +	
		5-6-7-8-9-10-11-12-13-14	—(10x)	Case was discharged from the Hospital.
		(1st 14 tests were made on successive days.)		
		5 days later, Nov. 13	+	
		Nov. 14, 15, 16, 18	—(4x)	
		Nov. 19 T.N. 1 Day	+	
		" 20 " 2 Days	—	
		" 21 3 "	+	
		" 22, 23	— —	
		" 25, 26	— —	
		" 27, 29, 30	— — —	
		Dec. 3, 4, 5, 6, 7	- - - - -	
		" 27, 30	— —	
Case 9	M.	T.N., 12 Days	+	Almost pure culture of typhoid bacilli. Case was discharged from Hospital a day or two later, before a second test was made.
		2nd Test—3 Months later	—	
		3rd " —3 " & 7 Days	—	

	Sex.	Temperature Normal.	Result.	Remarks.
Case 10	M.	1st Test—3 Days	+ (80 % of colonies were typhoid.)	Case was discharged from Hospital before a second test was made.
Case 11	M.	1st Test—21 Days	+	Two days later, case was discharged from the Hospital and no tests could be made.
Case 12	M.	1st Test—7 Days	+	Case was discharged from the Hospital without further tests.
Case 13	M.	1st Test—14 Days	+	Case was discharged from the Hospital without further tests.

The next two cases are given in full as follows:

Case 14. An insane woman who had a light case of typhoid in October, 1907:

	Result.
Nov. 6—1st examination of feces made when temperature normal 7 days	+
" 13	—
" 20, 27	+ +
Dec. 4	—
" 11, 18, 27	+ + +
1908	
Jan. 2, 8, 15, 22, 29	+ + + + +
Feb. 5, 12, 19, 26	+ + + +
Mar. 4, 11, 18	+ + +
" 25	—
Apr. 8, 15	+ +
" 29	—
May 6, 13, 20, 27	+ + + +
June 3, 10, 17, 24	+ + + +
July 1	—
" 6, 15, 22, 29–Aug. 9	+ + + + +
Aug. 12	—
" 19	+
" 27	—
Sept. 2, 9, 17	+ + +
" 24	—
" 30–Oct. 7, 14	+ + +
Oct. 22	—
" 28–Nov. 4, 11, 25	+ + + +
Dec. 8, 10, 24	+ + +
" 29	—
1909	
Jan. 6, 13, 26	+ + +
Total	60 examinations, 50 stools in which typhoid bacilli were found.

Case 15. An insane woman who had a light case of typhoid in September, 1907:

	Result.
1st stool tested Oct. 23, temperature normal 14 days	—
Oct. 31	+
Nov. 6	—
" 13, 20, 27	+ + +
Dec. 4	—
" 11, 18	+ +
" 27 & Jan. 1, 1908	—
——	— —
1908	+ + +
Jan. 8, 15, 22	+ + + +
" 29	+ + + +
Feb. 5, 12, 19, 26	+ + + +
Mar. 4, 11, 18, 25	— —
Apr. 8, 15, 29–May 6	+ + + + + +
May 3, 20	+ +
" 27–June 3, 10, 17, 24	—
July 1, 6	+ + + +
" 15	+ + + +
" 22, 29–Aug. 5, 12	—
Aug. 19, 27–Sept. 2, 9	+ + + +
Sept. 17	—
" 24, 30–Oct. 7, 14	+ + + +
Oct. 22	+ +
" 28–Nov. 4, 11, 25	— —
Dec. 8, 10	
" 24, 29	
——	+ + +
1909	——
Jan. 6, 13, 26	Total number of stools tested, 62, of which 49 were found to contain typhoid bacilli.

Typhoid found = +
Typhoid not found = —

The second set of cases which we have classed as post-typhoid was obtained from the New Jersey State Hospital at Trenton. During the summer and early fall, 1907, there had been an outbreak of typhoid in the institution. Five months later an examination was made of the stools of the inmates who had had typhoid at that time. There were 54 cases which were examined as follows:

	8 cases	1 stool examined
	31 "	2 stools "
	13 "	3 " "
	1 case	4 " "
	1 "	5 " "
Total	54	118

Two of these cases were found still harboring typhoid bacilli: cases 16 and 17. Case 16 died in October, 1908, but not from typhoid. Case 17 is still under observation.

		Result
Case 16	Stool tested 5 mos. after typhoid	+
	" " 6 " " "	+
	" " 7 " " "	+
	" " 8 " " "	+
Case 17	Stool tested 5 mos. after typhoid	+
	" " 6 " " "	—
	" " 7 " " "	—
	" " 8 " " "	—
	" " 1 yr. 10 mos. after typhoid	+

Cultures from the 17 cases in which stools were found to contain typhoid bacilli were tested for agglutination with an anti-typhoid horse serum as shown in the following tables. A culture of B. coli was also tested, as well as a laboratory typhoid culture Mt. S., which was used as control.

TABLE I

ANTI-TYPHOID HORSE SERUM

	Control of Broth Suspension.	1–2000	1–5000	1–10,000	1–20,000	1–40,000
B. Coli	—	—	—	—	—	—
Mt. S.	—	+ +	+ 1	+	+	± (—)
Isolation 1	—	+	+	±	+ (—)	1
“ 2	—	+	+	±	—	—
“ 3	—	+	±	± (—)	1	—
“ 4	—	+	+	±	1	—
“ 5	—	+ 1	+	+	±	± (—)
“ 6	—	+ +	+ +	+ 1	+	±
“ 7	—	+	+	±	±	1
“ 8	—	+	+	±	± (—)	1
“ 9	—	+	+	±	±	1
“ 10	—	+	±	±	± (—)	± (—)
“ 11	—	+	±	± (—)	1	1
“ 12	—	+	±	± (—)	—	—
“ 13	—	+	±	±	+	1
“ 14	—	+ +	+ 1	+	+	±
“ 15	—	+ +	+ +	+	+	± (—)
“ 16	—	+	+	+	+	±
“ 17	—	+ +	+ +	+ 1	+	±

+ + = Absolute agglutination.
+ 1 = Almost complete agglutination.
+ = Good agglutination.
± = Fair agglutination.
1 = Tendency.
— = No agglutination.

Each culture was also added to the anti-typhoid horse serum in sufficient amount to absorb the greater part of the agglutins. In every case the specific typhoid agglutin was largely absorbed, as much so as by our laboratory culture. The results with the last seven cultures are given below. The others were very similar.

Absorption of agglutinins in anti-typhoid horse serum by Isolation 11

Dilution of Filtrate.	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	C.
Mt.S	++	++	+1	+1	+	±	—	—
Isolation 11	+1	+1	+	±	1	1	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 12

Dilution of Filtrate.	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	C.
Mt.S	++	++	+1	+	±	±	1	—
Isolation 12	+1	+1	±	1	—	—	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 13

Dilution of Filtrate.	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	1–2560	C.
Mt.S	++	++	++	+1	+1	+	±	—	—
Isolation 13.	+	±	1	1	—	—	—	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 14

Dilution of Filtrate.	1–10	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	C.
Mt.S	+	±	±	1	1	—	—	—	—
Isolation 14.	+	±	1	—	—	—	—	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 15

Dilution of Filtrate.	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	C.
Mt.S	++	++	++	+1	+	1	—	—
Isolation 15	++	++	++	+1	+	±	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 16

Dilution of Filtrate.	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	1–2560	C.
Mt.S.......	++	++	+	±	1	—	—	—	—
Isolation 16.	++	+	±	1	1	—	—	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 17

Dilution of Filtrate.	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	1–2560	C.
Mt.S.......	++	++	++	+1	+	±	1	—	—
Isolation 17.	++	++	++	+1	+	—	—	—	—

The case of the typhoid carrier, Mary M———, which we have mentioned above, has been under observation since March, 1907. The bacteriological examinations of the feces for the first ten months have already been reported; the results of those made in 1908 are as follows:

MARY M.—YEAR 1908

January 2, 8, 15		Typhoid present.
January 22, 27		Typhoid present.
February 5		Almost pure culture typh.
February 12		25% typhoid colonies.
February 19		7-8 typhoid colonies per plate.
February 26, March 4, 11, 18, 25	"few" colonies	Typhoid present.
April 1, 9, 15, 29		Typhoid present.
May 6		Typhoid present.
May 20		No typhoid found.
May 27, June 3, 10, 17, 24, July 1		Typhoid present.
July 6, 15		No typhoid found.
July 22		Typhoid present.
July 29		No typhoid found.
August 5, 19, 27		Typhoid present.
September 2, 7		Typhoid present.
September 17		No typhoid found.
September 24		Typhoid present.
September 30		No typhoid found.
October 7		20% typhoid.
October 14, 22, 28		Typhoid present.
November 4, 11		4-5 typhoid colonies per plate.
November 18, 25		10% typhoid colonies.
December 2		30% typhoid colonies.
December 10		40% typhoid colonies.
December 7		2-3 colonies typh. per plate.
December 17		6-7 typhoid per plate.
December 23		3-4 typhoid per plate.
December 30		3-4 typhoid per plate.

Normal Cases Having No Typhoid History

Of the normal cases examined, there were 188, one stool being tested in each case. No typhoid bacilli were found.

REPORT OF BACTERIOLOGICAL EXAMINATION OF CROTON TAP WATER FOR THE YEAR 1908

By W. Carey Noble

Croton tap water at East 16th Street was plated in agar and tested for the presence of colon bacilli once a week during the year. The colony count at 37° C. and 24° C. and the result of the presumptive test were as follows:

	1 c.c. Plated in Agar 37° C. for 24 hours.	At 24° C. 72 hours.	Quantity of Water Containing Colon Bacilli as Shown by the Presumptive Test (Lactose peptone water).
Jan. 3, 1908	24 colonies	122 colonies	1 c. c.
“ 7	27 “	163 “	10 “
“ 15	84 “	159 “	1 “
“ 21	73 “	218 “	1 “
“ 30	18 “	34 “	10 “
Feb. 4	42 “	137 “	10 “
“ 13	37 “	84 “	10 “
“ 19	52 “	136 “	9 “
“ 25	106 “	127 “	1 “
Mar. 3	34 “	306 “	1 “
“ 10	15 “	162 “	1 “
“ 17	20 “	63 “	10 “
“ 24	18 “	46 “	10 “
“ 31	27 “	132 “	0.1 “
Apr. 6	22 “	115 “	10 “
“ 15	144 “	1370 “	0.1 “
“ 21	60 “	321 “	0.1 “
“ 28	90 “	167 “	0.1 “
May 5	32 “	134 “	1 “
“ 12	67 “	135 “	1 “
“ 19	62 “	70 “	0.1 “
“ 26	59 “	920 “	0.1 “
June 3	30 “	96 “	0.1 “
“ 9	31 “	97 “	0.1 “
“ 17	82 “	152 “	0.1 “
“ 24	28 “	124 “	1 “

	1 c.c. Plated in Agar 37° C. for 24 hours.	At 24° C. 72 hours.	Quantity of Water Containing Colon Bacilli as Shown by the Presumptive Test (Lactose peptone water).
July 1	48 colonies	276 colonies	1 c.c.
" 8	40 "	90 "	0.1 "
" 15	75 "	337 "	0.1 "
" 22	59 "	320 "	0.1 "
" 29	139 "	432 "	0.1 "
Aug. 5	63 "	171 "	0.1 "
" 12	1289 "	3160 "	0.01 "
" 20	137 "	268 "	0.01 "
" 26	178 "	316 "	0.01 "
Sept. 2	217 "	640 "	0.01 "
" 9	368 "	283 "	0.01 "
" 16	130 "	177 "	0.01 "
" 24	106 "	238 "	0.1 "
" 30	82 "	139 "	0.1 "
Oct. 8	102 "	201 "	0.1 "
" 15	327 "	710 "	0.01 "
" 20	115 "	211 "	0.1 "
" 29	50 "	190 "	0.1 "
Nov. 4	28 "	132 "	0.1 "
" 12	97 "	228 "	1 "
" 18	44 "	121 "	0.2 "
" 25	90 "	186 "	0.02 "
Dec. 1	25 "	60 "	0.1 "
" 10	38 "	77 "	0.05 "
" 16	10 "	46 "	1 "
" 24	90 "	156 "	10 "
tank on roof			
Dec. 31	1030 "	1090 "	0.02 "

THE QUANTITATIVE CHANGES IN THE PROTEINS IN THE BLOOD PLASMA OF HORSES IN THE COURSE OF IMMUNIZATION

BY

Edwin J. Banzhaf and Robert B. Gibson

When an animal is immunized against bacterial toxins (Seng,[1] Joachim,[2] Atkinson,[3] Ledingham[4]) and against foreign proteins (Moll[5]), there result certain characteristic quantitative changes in the proteins of the blood plasma. The content of serumglobulin is strikingly increased, even up to double the normal amount; at the same time there is a diminution in the serumalbumin. Similar changes have been said to occur during starvation,[6] Moll, however, has denied that the inanition is the cause of the globulin increase observed in precipitin sera.

Atkinson[7] showed that this serumglobulin increase is in some degree proportional to the antitoxic (diphtheria) potency of the serum. His analyses give figures for the protein precipitable on saturation with magnesium sulphate; this "globulin"[8] paralleled more or less the gross changes in the antitoxic potency in the serum for the one horse so examined. With his other observations on the increased "globulin" and the antitoxic content of antitoxic serum, a more than incidental relationship between the serumglobulin increase and the antitoxic content is suggested.

In a paper published while the present investigation was under way, Ledingham[9] gives results of the determination of the total protein, the serumglobulin and the serumalbumin in the course of immunization against diphtheria toxin. The serum was obtained at short intervals from two horses and a goat. With one horse, which failed to yield a high grade antitoxin, the serumglobulin content of the serum was not essentially increased over the normal. The other horse gave ultimately a 650 unit serum; the serumglobulin, here, had progressively increased along with the antitoxic potency. A goat serum which had reached 40 units showed a rise in both the serumalbumin and (to a much less degree) of the serumglobulin over the normal figures. From

Ledingham's results on the two horses, a relation between the development of the antitoxic properties and the increase in the serumglobulin content is indicated.

The observations of Atkinson and of Ledingham, so far as we are aware, are the only determinations of the quantitative relation of the serumglobulin content and antitoxic potency throughout the course of immunization. The subject is of extreme importance because of the constant association of the anti-substance with the serumglobulin.[10] The serumglobulin increase in this protein during immunization may actually represent the accumulation of protective substances; at least if not identical, the antitoxin may be in some loose chemical combination with the protein. Mellanby[11] recently states that even the diphtheria antitoxin is actually a part of the serumglobulin (albumin$^{\alpha}$).

The poverty of the data as to the blood changes during immunization make additional and more conclusive experiments desirable when the significance of the problem as to the chemical nature of the protective substances is considered. In the present paper we present the results of a large number of analyses upon the protein changes in the plasma of 11 horses subjected to immunization.

We have followed quantitatively the protein changes in the sodium oxalate plasma in a series of 11 horses. At first these were undergoing simultaneous immunization, under the direction of Dr. Wm. H. Park, to both tetanus and diphtheria toxins; the horses were subsequently kept on the toxin to which they responded best individually as regards antitoxin production.[12] The first few injections were made with toxin after the administration of antitoxin, as is the custom at the Research Laboratory. The immunization was forced, i. e., the toxins were administered in relatively large doses, usually in weekly injections. The horses were bled for serum production at about 8, 10 or 14 day intervals, when a maximum antitoxic value was attained as indicated by earlier test bleedings of about 500 c.c. of blood. The regular bleedings for serum production (six to ten liters of blood) were instituted, accordingly, only *after* maximum antitoxic (and protein) changes had occurred, as shown in the tables. The variations during the period of increasing antitoxic potency, therefore, have not been influenced by severe hemorrhage.

Suitability of Individual Horses for Antitoxin Production

As has long been known, certain young and healthy horses seem refractory for the production of diphtheria or tetanus antitoxins; again, certain horses will yield a much more potent antitoxin than others treated in an exactly identical manner. This idiosyncrasy is strikingly brought out in the behavior of the animals used in the present experiments. Thus six (319, 322, 323, 326, 327 and 328) of the eleven horses reacted to diphtheria but not to tetanus toxin; the plasma obtained from these six, at maximum potency, was 550 units per c.c. or above, except in the case of 327, where the strength was 400 units. One horse (320) gave only a potent serum against tetanus while being simultaneously refractory for the production of diphtheria antitoxin. Two horses (321 and 324) failed to react vigorously on immunization with either toxin; another (325) responded in some degree to both. Horse 318 was immunized to a high degree (600 units) to diphtheria toxin; transferred to tetanus, it yielded a very potent serum (300 units), and when returned to the diphtheria service nearly the original potency was re-established. There is then an individual adaptation on the part of the horse for the production of certain antitoxins; for other toxins the horse may be almost completely refractory.

MAXIMUM ANTITOXIN POTENCIES IN UNITS

Horse.	Dip.	Tet.
318	600	300
319	600	4
320	50	90
321	150	2
322	625	5
323	850	5
324	125	1
325	300	25
326	600	1
327	400	1
328	550	1

Note.—Maximum potencies for diphtheria and tetanus antitoxins are, of course, not always here coincident.

Protein determinations were made in duplicate by heat coagulation and weighing on the oxalate plasma, as follows:

1. The total protein.
2. The total protein less the fibrinogen.

The protein in an aliquot of the filtrate from the fibrinogen, precipitated at 2.9 saturation[13] ammonium sulphate and at an ultimate dilution (when precipitated) of tenfold the original volume of plasma taken for the fractioning.

3. The serumalbumins.

The protein in an aliquot of the filtrate at 5.0 (half saturation) ammonium sulphate at a dilution of the plasma, when precipitated, of tenfold the original volume of plasma taken.

4. (a) The serumglobulins soluble in saturated NaCl solution.

Separated by direct solution in saturated NaCl solution of the proteins precipitated at half saturation ammonium sulphate at an ultimate plasma dilution of 1 : 10.

(b) Or the protein in solution on saturating the plasma with NaCl.

The several times diluted plasma was saturated with the dry salt and made up to ten times the volume of plasma taken with saturated NaCl solution. Determinations were made on aliquots of the filtrates. This separation was more commonly used than the preceding.

5. The "pseudoglobulin" plus the serumalbumin.

The protein of aliquots of the filtrate from the plasma precipitated at third (3.3) saturation ammonium sulphate and at ultimate dilutions of 1.5, 5 and 10 times the volume of the plasma used.

The volume of the precipitates obtained in the above determinations was so small that but little error should be obtained in employing aliquots of the filtrates as above for the analyses. In the precipitation of the "euglobulin" at 1 : 1.5 dilution, however, such error must influence the results somewhat; even here we were never dealing with a precipitate from 10 c.c. of oxalate plasma of over 0.5 gm. in a volume, when precipitated, of 15 c.c. Inasmuch as the density of proteins is greater than water, and the volume of solvent is increased somewhat by their solution, the error in the analytical results must certainly be less than 3%, an error perfectly negligible in the interpretation of the figures for the "euglobulin" at the dilution of the plasma of 1 :1.5.

From the analytical data so obtained, the content of the plasma in the following protein constituents is available.

The total protein.

Fibrinogen.

Serumalbumins.[14]

Serumglobulins.[14]

Serumglobulin, insoluble in saturated NaCl solution.

Serumglobulin, soluble in saturated NaCl solution.

"Euglobulin" precipitated at ultimate dilutions of the plasma of 1 :1.5, 1 :5 and 1 :10.

"Pseudoglobulin," similarly precipitated.

Because of the large amount of analytical data obtained, it does not seem advisable to go into details of the variations in each of the 11 horses examined. Certain interesting and important *general* features of the results will be discussed in full and example given from the material at hand. The conduct of the experiment and the results of the analyses are more fully given in the tables at the end of this article. Some graphic representations of the protein and antitoxic changes are also reproduced.

The characteristic serumglobulin increase in the plasma during immunization was observed *in all the eleven horses.* Certain characters of this increase may be discussed.

The maximum increase in the serumglobulin was usually coincident with the maximum antitoxic potency of the plasma. Two exceptions were noted (horses 320 and 324). The maxima were attained in the seventh to ninth week of immunization, but both antitoxin and serumglobulin may be diminished even at the time of the first antitoxin bleeding[15] (319, 322, 323, 325).

The curves of the serumglobulin increase and the development of the antitoxic potency were not otherwise parallel. The maximum serumglobulin content in two horses (320 and 324) was actually reached previous to the highest antitoxic potency.

After immunization for a period of 39 days, horse 320 gave a plasma of 20 antidiphtheria units per c.c., the serumglobulin content of which was increased 42.9% over the normal. The plasma obtained on the 59th day tested up 35 antidiphtheria and 2 antitetanus units; the serumglobulin had increased to its maximum of 87.6% over the normal. Twenty-eight days later the potency had risen to 50 units for each antitoxin, while the serumglobulin increase had fallen to 69.7%. Subsequently, with a further rise in the tetanus antitoxin to 90 units, the serumglobulin had diminished to 48.4% over the normal. For the second horse, 324, antitoxic potencies of 25,125 and 125 units were accompanied by a serumglobulin content above the normal of 59.3%, 48.5% and 38.9% respectively.

With the exception of horses 318 and 319, on which we have no "intermediate" analyses, the other animals all showed a marked increase in the serumglobulin content, *preceding* the development of the antitoxic properties.

For example, 32 days after the combined immunization was started, horse 322 yielded a plasma which tested 50 units of diphtheria antitoxin per c.c. and in which the serumglobulin had been increased 44.8%; 35 days later the plasma tested 625 units and the serumglobulin increase was 90.6%. The antitoxic potency, therefore, had been brought up over 12 times while the serumglobulin increase had been only double during the same period. Similarly, the serumglobulin in the plasma of horse 325 had increased 40.7% after 27 days immunization, the potency being just 5 units; on the 54th day the serumglobulin increase was 90.2%, while the antitoxic potency had risen to 600 units of diphtheria and 1 unit of tetanus antitoxin.

While the greatest rise in the serumglobulin was usually coincident with maximum antitoxic potency, as already pointed out, the extent of this increase in the serumglobulin was practically independent of the antitoxic potency when the results on more than one horse were contrasted. There may be, then, no relation between the absolute or percentage increase of the serumglobulin and the antitoxic potency in the plasma of different horses. The increase in the serumglobulin of refractory horses may surpass that in the plasma of some of those yielding a high antitoxin. The greatest absolute increase in serumglobulin (4.40 gms.), however, was observed in the plasma of the horse (323) which supplied the strongest antitoxin serum of the series. In contrast, the refractory horse 321 showed an absolute increase in the serumglobulin of 4.07 gms. per 100 c.c. plasma.

For horse 318 this increase was 41.1% (2.01 gms. per 100 c.c. plasma); for 319, treated in exactly the same manner during immunization and yielding a plasma of the same potency (600 units diphtheria), the increase was 86.3% (3.66 gms.). Horse 321 showed an increase of 87.6% (4.07 gms.) in the serumglobulin when supplying a plasma of maximum potency of only 150 units per c.c.; this horse failed to react well to the toxin not only as regards antitoxin production, but died subsequently of toxemia when the immunization was continued. Horse 323 gave a maximum of 850 units (diphtheria) and the serumglobulin had been increased 95.6% with the greatest absolute increase (4.40 gms.) we have observed. Horse 328 yielded a 550 unit plasma, the serumglobulin increase being 113.9% (4.00 gms.). The refractory horse 324 is especially interesting. At the third bleeding (5/31/07) a potency of 125 units (diphtheria) and a serumglobulin increase of 48.5% (2.69 gms.) were found; 19 days later (6/19/07) the antitoxic strength was unchanged, but the serumglobulin content had fallen until the increase was only 38.9% above the normal. A similar change was observed for horse 326, where the serumglobulin maximum fell from 7.61 gms. (4/30/07) to 5.92 gms. without change of antitoxic strength; the results here, however, are complicated by the "full" bleeding.

SERUMGLOBULIN INCREASE AT MAXIMUM ANTITOXIC POTENCY

Horse.	Potency.		Serumglobulin.	Increase.
	Dip.	Tet.	Gms per 100 c.c. Plasma.	Per cent.
318	600	1	2.01	41.1
319	600	1	3.66	86.3
320	50	90	2.19	46.2*
321	150**	2	4.07	87.6
322	625	5	3.56	90.6
323	850	5	4.40	95.6
324	125**	1(?)	2.69	48.5*
325	300	4	3.22	89.1
326	600	1	3.61	90.2
327	400	1	2.87	102.1
328	550	1	4.00	113.9

* Maximum serumglobulin increase preceded maximum antitoxic potency.
** Refractory horse.

When the serumglobulin had reached a maximum with maximum antitoxic strength, the protein diminished with the decrease in potency even before regular bleedings were established. It was the practice in the Research Laboratory to bleed the horses for antitoxin production when test bleedings indicated a high or maximum potency. In some instances the horses reached a maximum, and the antitoxic potency had fallen somewhat by the time the first regular "full" bleeding was instituted. This diminution in potency was accompanied by a lowering of the serumglobulin from the maximum content. This fall, however, seems to have had no quantitative relation to the degree of the potency changes, which in some instances were very slight. Accordingly the lowering of the serumglobulin is probably identical with the submaximal fall in serumglobulin coincident with the highest potency in the plasma of horses 320 and 324, and is characteristic in itself and more or less independent of the antitoxin variations.

For instance, horse 319 gave a maximum of 600 units diphtheria antitoxin and 1 unit of tetanus. The serumglobulin increase was then 3.66 gms. per 100 c.c. of plasma. With a change in antitoxic potency to 550 units of diphtheria and 2 of tetanus antitoxin (bleeding 4/2/07), the serumglobulin increase had fallen to 2.81 gms. Thus for a decrease in the antitoxin properties of about 1-12, the serumglobulin increase had been reduced ¼. A fall in the potency of the plasma of horse 322 from 625 units of diphtheria antitoxin and 5 of tetanus, to 500 units of diphtheria (5/3/07) cut down the increase in the serumglobulin from 3.56 to 3.01 gms. Here, then, a fall of 1-24 in potency caused a drop in the increase of serumglobulin of a trifle less than 1-6. Similarly when the plasma of horse 323 fell from a maximum of 850 units of diphtheria and 5 of tetanus antitoxin (4/29/07) to 550 units of diphtheria (5/10/07), the drop of over 35% in the potency was accompanied by a curtailment of 22.5% in the increase of the serumglobulin over the normal.

We have already shown that during immunization the serumglobulin increase tends to precede the development of the antitoxic potency; that the two reach a maximum, usually coincident, and that with the fall in potency, or even before, the increase in the serumglobulin is diminished to greater degree than the potency change. This last decline continued for one-third "full" bleedings, apparently irrespective as to whether the antitoxic properties were maintained or not. Subsequently there was a slight increase in the serumglobulin.

For example, at the first "full" bleeding (4/30/07) of horse 326, the 8 liters of plasma obtained tested 600 units per c.c., and the content in serumglobulin 7.61 gms. per 100 c.c. On the next "full" bleeding (5/10/07) the serumglobulin content of the plasma, of the same potency, had fallen to 5.92 gms. We are not dealing here with the effects of dilution, through bleeding, because the serumalbumin has been increased from 1.16 to 1.44 gms. per 100 c.c. plasma. Eight days later (5/18/07) the potency was still 600 units; the serumglobulin content had increased from 5.92 gms. to 6.96 gms. per 100 c.c. of plasma, while the serumalbumen had fallen from 1.4 gms. to 0.98 gms. Practically the same fall is shown by the analyses in the plasma of horses 319, 320, 323 and 325. For horse 318 similar variations occur, but because of the double immunity here it is impossible to give a comparative value to the potency variations. The latter rise is well brought out in the figures for horses 318, 319, 323 and 325.

The serumglobulin content was maintained high and fairly constant during active antitoxin production. At times there was a tendency for the serumglobulin to follow quantitatively the potency variations; at other times, the increase was independent of the antitoxin changes (horse 326, 5/19/07 and 6/13/07). With horse 325, for instance, the serumglobulin did not increase for a rise in potency from 150 to 250 units (7/2/07 to 9/17/07) in the course of the regular bleedings, but was diminished from 6.09 to 5.39 gms. per 100 c.c. plasma. Subsequently when the lowering of the serumglobulin content was accompanied by a corresponding drop in the antitoxic potency, interruption of the regular "full" bleedings failed to check the diminution of either potency or serumglobulin.

In the course of immunization, then, there is a marked increase (even over 100 per cent.) in the serumglobulin content of the blood plasma. This increase tends to precede the development of maximum antitoxic potency and is quantitatively independent of the latter. With a diminution of the potency, the serumglobulin increase is reduced, with a tendency for this change to precede the antitoxic variation; then after a slight rise, the content of serumglobulin is maintained at a fairly constant level in spite of repeated "full" bleedings at frequent intervals. The serumglobulin now may or may not be independent of incidental variations in the antitoxic potency. As a rule, it does parallel to some extent the pronounced changes in the antitoxic properties of the plasma.

Changes in the "euglobulin" must be considered because the increase during immunization of the serumglobulin has been stated by Joachim[16] to take place in this fraction. Some discussion of the chemistry of the serumglobulin fractions is desirable.

The serumglobulin is commonly stated to consist of at least two different proteins, termed the "euglobulin" or true globulin, and a more soluble protein, the "pseudoglobulin." The "euglobulin" is supposed to be insoluble in pure water, and accordingly precipitable on dilution or dialysis; it is thrown down also by slight acidification. The "pseudoglobulin" belongs by definition to the group of true albumins, i. e., it is soluble in pure water. It has received the title "pseudoglobulin" probably because the earlier differentiation was made on the basis of precipitation on saturation of its solution with magnesium sulphate. The separation of the two proteins then should be relatively easy by the dialysis method. It had always been our experience, however, that the ordinary globulin precipitation methods, with the exception of the salting out reactions, are practically without effect on horse serum. On dilution, dialyzation[17] or slight acidification of the natural or previously neutralized (phenolphthalin) horse serum only a very small amount of precipitate is obtained. In practice, investigators have commonly used the fractional precipitation by salting out with ammonium sulphate. A half volume of the saturated ammonium sulphate solution is added to the serum; the precipitate is the "euglobulin." The separation is not sharp at all. The filtrate contains the serumalbumin and "pseudoglobulin"; the latter can be thrown down by the further addition of saturated ammonium sulphate solution to "half" saturation, i. e., until the mixture contains equal volumes of serum and the salt solution. The "euglobulin" may be further purified by redissolving the precipitate in a measured quantity of water and again adding a half volume of the saturated ammonium sulphate solution. In current writings the identity of this "fractioned" euglobulin has been confused with the slight precipitate obtained on dialysis, dilution or slight acidification in spite of the early work of Freund and Joachim.[18]

The differentiation of the serumglobulin into "eu" and "pseudo" fractions by the ammonium sulphate salting out was further established by the result of E. P. Pick.[19] This investigator fractioned various antitoxic, agglutinative and other sera and differentiated the antibodies chemically according to the fraction with which these were precipitated. Others[20] have also similarly fractioned individual antisubstances of various kinds and reported with which fraction their own particular body was associated. One of us (with Dr. K. B. Collins)[21] has already shown that no such separation of the agglutinins in polyagglutinative sera can be made, and has failed to verify Pick's results on antitoxic sera.[22] We[23] have, however, found that the more soluble of several fractions of the "pseudoglobulin" are richest in antitoxin per gm. protein. By employing the ammonium sulphate fractioning, Ledingham,[24] like Joachim, found that the increase in serumglobulin affected the "euglobulin" more than the "pseudoglobulin" fraction, i. e., the great increase in the serumglobulin took place in the non-antitoxic portion. Ledingham's interpretation of his results, as regards this point, seem inconclusive, for he does not appear to grasp the significance and understand the limitations in the salt fractioning of proteins.

We have already stated in an earlier paper that precipitation limits in the salting out of proteins are characteristic only in so far as they should be interpreted as well from the standpoint of the *solubility of the protein in certain amounts of the salt solution of a definite concentration.* Precipitation occurs because the solvent is saturated with the protein, i. e., it can hold no more in solution; with a greater volume for the solvent more precipitated protein will go into solution. Moreover, other kinds of proteins remaining in solution probably affect the precipitation of the less soluble forms. Haslam's[25] criterium of purification calls for repeated precipitation at the same volume until the organic N. in the filtrate is constant.

The observations associating the increase serumglobulin with the questionable "euglobulin" fraction have been made by direct precipitation of the serum with half its volume of saturated ammonium sulphate. Where precipitated for purification, the protein has usually been redissolved to the original volume and the same amount of ammonium sulphate solution added as before. Inasmuch as the serumglobulin content may have been doubled as the result of immunization, would not a great part of this increase be found naturally in the "euglobulin" fraction, since solubility of the protein must be considered? The precipitate obtained at "one-third" saturation is not free from antitoxin.[26] It may contain the greater part of it when the undiluted serum is precipitated by the addition of a half volume of saturated ammonium sulphate solution.

Ten c.c. of the normal plasma were precipitated at one-third saturation (3.3) by the direct addition of 5 c.c. of the saturated ammonium sulphate solution; again 10 c.c. of the plasma were diluted 33 1-3 c.c. and precipitated by the addition of 16 2-3 c.c. of the saturated ammonium sulphate solution, and finally, a third 10 c.c. of the plasma were diluted to 66 2-3 c.c. and precipitated with 33 1-3 c.c. of the saturated salt solution. We had then ultimate dilutions to 15, 50 and 100 c.c. The precipitates were allowed to stand for some hours, with occasional shaking, to permit mechanically precipitated material in part to return to solution. The "euglobulin" at the three dilutions was determined as outlined earlier in the paper.

In such an experiment with horse 319 the normal total serumglobulin (4.24 gms. per 100 c.c. plasma) was found to include 2.43, 0.79 and 0.48 gms. of "euglobulin," according as the dilution of the precipitated plasma was 1:1.5, 1:5 or 1:10. When similarly fractioned at the height of immunization the three "euglobulins" were 5.39, 1.78 and 0.98 gms. at the three respective dilutions. Of the increase of 3.66 gms. in the total serumglobulin, about four-sevenths (2.96 gms.) would be contained in the "eu" fraction at the 1:1.5 dilution; 0.99 gms., or about two-sevenths, would be accounted for at the 1:5 dilution, and but one-seventh at the 1:10 dilution. The precipitations were probably influenced by the lowered serumalbumin content of the antitoxic plasma. It will be noted that the "euglobulins" obtained at the three ultimate dilutions have about the ratio 5:2:1 for almost any of the plasma samples analyzed.

A similar behavior on the part of the "euglobulin" at the several dilutions was observed in the analyses for each horse. Most of the "euglobulin" determinations during the routine "full" bleedings were made at the 1:10 dilution only, and these figures probably most fairly represent the content of this protein if such actually exists. Throughout the whole series of experiments, the per cent. increase in the "euglobulin" tended to parallel that of the total serumglobulin.

Because of the unsatisfactory characterization of the "eu" and "pseudo" globulin by the ammonium sulphate fractioning, it seemed of interest to differentiate in some modified way the relative serumglobulin quantitative changes. We have accordingly determined that portion of the serumglobulin precipitated on saturation with sodium chloride. Freund and Joachim state that saturation with NaCl precipitates all the "euglobulin," but that this includes only a part of the product obtained at 3.3 saturation ammonium sulphate. We may consider that we have as sharp a differentiation as possible between the two serumglobulins; of course, the individuality of each cannot yet be asserted. The NaCl separation probably more truly represents the common conception of the "euglobulin" and "pseuglobulin," though the ammonium sulphate fractioning has usually been employed.

A glance at the table of analyses for any bleeding will show that the serumglobulin precipitated on saturation with NaCl and the ammonium sulphate "euglobulin" are quantitatively different. Accepting for the "euglobulin" the figures obtained with the $(NH_4)_2SO_4$ at 1:10 dilution, we found that the sodium chloride gave a precipitate of serumglobulin in the normal plasma that was from two to three times greater than the corresponding fractioned protein.

The changes during the immunization in the saturated NaCl precipitated serumglobulin are significant. With the rapid rise in the serumglobulin content after immunization has been started, there was observed a very considerable increase in the portion of the serumglobulin precipitated on saturation with NaCl. This rise is shown in the record for horses 322, 323 and 327, but was not observed with the others.[27] Subsequently there was a sharp fall in the NaCl precipitable serumglobulin. This decrease was maintained far below the absolute and per cent. normal, and tended to vary inversely with the total serumglobulin content; the behavior in this respect is exactly the opposite of the "euglobulin" fractioned with ammonium sulphate, and parallels closely the changes in the serumalbumin.

For example, the serumglobulin precipitated on saturation with NaCl, in the normal plasma of horse 325 was 0.91 gm. per 100 c.c. plasma of 24.7% of the total serumglobulin (3.68 gms.) with the preliminary rise of 36.7% in the total serumglobulin, the NaCl "euglobulin" had increased over 60% above the normal (1.54 gms. per 100 c.c. plasma) and to 30.6% of the total serumglobulin. The serumglobulin precipitable on saturation with NaCl then sharply fell; with the coincident maxima of antitoxic potency and total serumglobulin content, this NaCl "euglobulin" was only 0.49 gms. (per 100 c.c. plasma) or 7.1% of the total globulin. At the same time the $(NH_4)_2SO_4$ "euglobulin" had increased from a normal of 0.42 gms. to 1.00 gm. per 100 c.c. plasma.

The variations in the content of serumalbumin, during immunization, have already been described in detail by Atkinson and by Ledingham. We have also found the curious and characteristic diminution in the content of the plasma in this protein. The minimum content is reached as a rule somewhat subsequent to the time of the serumglobulin and antitoxic maxima; it occurred usually during the period of the routine bleedings. While the greatest increase observed in the total serumglobulin was about 114 per cent., the serumalbumin may be reduced to less than 20 per cent. of the normal—a diminution from 2.80 gms. to 0.52 gm. being observed in the case of horse 319. The course of the plotted curve of the serumglobulin then was roughly the reverse of that of the total serumglobulin; the serumalbumin, however, tended to return to the higher level when the horse had been in use for several months as an antitoxin producer (319, 323, 325).

The behavior of the NaCl "euglobulin" and the serumalbumin suggests that these proteins are sacrificed in order to compensate for the great absolute increase in the more soluble serumglobulin with which the anti-properties are associated. Possibly because of the increasing viscosity, there must be a physiological limit to the amount of protein which can be carried in the blood plasma. Some 80 odd determinations have indicated that this limit is attained when the protein content is between 10 and 11 gms. per 100 c.c. plasma.*

The changes in the fibrinogen were often sudden and there was no apparent association with antitoxic potency, serumglobulin content or with bleeding. Analyses gave figures for fibrinogen of from 0.21 gm. to 1.0 gm. per 100 c.c. plasma, with variations in the individual horse of about 0.5 gm.

SUMMARY

Gravimetric determinations were recorded for the total and several individual proteins (in the sodium oxalate plasma) fractioned with ammonium sulphate and sodium chloride. At precipitation, the plasma salt mixture had been diluted to a final volume of 10 times the amount of plasma employed. Coagulations were on aliquot portions of filtrates, and the individual protein constituents (except serumalbumin) were calculated by difference.

The 11 horses had been subjected to simultaneous immunization against diphtheria and tetanus toxins, each horse being subsequently continued on the toxin to which it responded best. Test bleedings of about 500 c.c. only were made until maximum antitoxic potency (with almost coincident greatest variation in the contents of the several proteins) had been attained; routine bleedings of 4 to 10 liters for antitoxin production were then instituted.

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* Moll[28] has suggested that this serumalbumin is chemically transferred over into the serumglobulin during immunization. This statement is based on his heat and alkali transformation of albumin into globulin (?). Are we to accept, however, that the NaCl "euglobulin" might similarly be changed over into the more soluble serumglobulin?

The two refractory, one medium and the eight horses yielding a highly potent antitoxic plasma, all showed an increase of from 40 to 114 per cent. in the total serumglobulin. For the refractory animals, this increase was 59.3 and 87.9 per cents. In one refractory and one high horse, the serumglobulin maximum preceded the highest concentration in antitoxin. In seven of the horses, the greatest increase in the total serumglobulin was coincident with the maximum antitoxic potency. The serumglobulin increase, however, tended relatively to precede that of the antitoxin. In the two other horses, both maxima also were observed together; no "intermediate" bleedings were made in these two instances. The greatest absolute increase in the serumglobulin was observed in the most potent plasma obtained in the series; the second place, however, went to a refractory horse. Subsequent to the maxima, the serumglobulin content was maintained at high concentration, in spite of repeated bleedings; it then only roughly paralleled the antitoxic variations in the plasma of the individual animals.

At dilutions of the plasma in the precipitated mixtures of 1:1.5, 1:5 and 1:10, the ammonium sulphate "euglobulin" fraction amounted to about 67-70, 20-24 and 10-15 per cents., respectively, of the total serumglobulin in both the normal and the antitoxic plasma. In an 850 unit plasma an increase in the "euglobulin" over the normal per cent. was observed, but the high content of protein in this plasma probably influenced the precipitation limits. The influence of the protein concentration is indicated by the different percentages for the "euglobulin" obtained for the three dilutions of 1:1.5, 1:5 and 1:10. The "euglobulin" then was not increased to a greater extent than the "pseudoglobulin," as the result of immunization, as has at times been maintained.

The "euglobulin" precipitated by saturating the plasma with NaCl (at ultimate dilution of the plasma 1:10) was much greater in normal plasma than the $(NH_4)_2SO_4$ "euglobulin" at the same dilution. There was a tendency in early immunization for this NaCl "euglobulin" to increase along with the total serumglobulin; it rapidly diminishes, however, until at the height of immunization and maximum serumglobulin concentration, it may have reached less than half the normal absolute amount.

The serumalbumin was diminished a third to a half the normal along with the serumglobulin increase. Subsequent to the antitoxic and serumglobulin maxima, figures as low as a fifth the original serumalbumin content have been noted.

It is suggested that this diminution of the NaCl "euglobulin" and the serumalbumin is a physiological compensation for the greater viscosity of the plasma, because of the increase in the more soluble serumglobulins.

No characteristic alteration in the fibrinogen of the plasma was observed during immunization. Individual variations up to 0.5 gm. per 100 c.c. plasma have been regarded.

The influence of repeated bleedings does not essentially influence the protein changes induced by immunization. These remarkable regenerative processes are worthy of note.

The results of our investigation indicate that in "forced" immunization, the same characteristic quantitative changes can occur in the blood proteins of both refractory horses and of those yielding a highly potent antitoxic plasma. We cannot conclude, however, that the serumglobulin increase does not represent an accumulation of antitoxin, at least in part; it is possible that other antibodies may be formed either prior to or along with the specific antitoxin[29] and that these may constitute a portion of the increase in the more soluble serumglobulin with which protein such substances are associated.

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[1] Seng: *Zeitschr. f. Hyg.* xxxi, p. 513, 1899.

[2] Joachim: *Arch. f. d. ges. Physiol.*, xciii, p. 558, 1903.

[3] Atkinson: *Journ. of Exper. Med.*, v, p. 67, 1901.

[4] Ledingham: *Journ. of Hygiene*, vii, p. 65, 1907.

[5] Moll: *Zeitschr. f. exp. Path. u. Ther.*, iii, p. 325, 1906.

[6] Glaessner: *Zeitschr. f. Exp. Path. u. Ther.*, ii, No. 1, 1905.

[7] Atkinson: *Journ. of Exper. Med.*, v. p. 47, 1901.

[8] Under the classification of the proteins adopted by the American Physiological Society and the American Society of Biological Chemists, the greater part of the so-called serumglobulins are, at least for the horse, to be regarded as albumins.

[9] Ledingham: *Loc. cit.*

[10] With the more soluble or "albumin" type of the serumglobulin.

[11] Mellanby: *Proc. Royal Soc.*, B, lxxx, p. 399, 1908.

[12] It was originally Dr. Park's intention to report this immunization experiment in detail. The present paper contains a description of the experiment and the results only in so far as is necessary to make our own report complete.

[13] The degree of saturation, as here expressed, indicates a concentration in 10 c.c. of the precipitated mixture of 2.9 c.c. of saturated ammonium sulphate solution. The misconceptions that arise from the use of "per cent. saturation" and even from "third saturation" and "half saturation" have already been discussed in a previous paper. Cf. Banzhaf and Gibson: *Journ. of Biol. Chem.*, iii, p. 254, 1907.

[14] By "serumalbumins" is meant the protein material commonly known as such and consisting probably of more than a single individual substance. Similarly, the "serumglobulins" refer to the remaining proteins of the plasma, excepting the fibrinogen.

[15] The analyses for the plasma obtained at the first "full" bleeding are discussed with the earlier or "test" bleedings inasmuch as the horses had not previously been subjected to the severer hemorrhage.

[16] Joachim: *Loc. cit.*

[17] Long continued dialysis may lead to denaturalization and precipitation of otherwise soluble proteins, more than 48 hours is hardly necessary for the separation of typical albumins and globulins.

[18] Freund and Joachim: *Zeitschr. f. physiol. Chem.*, xxxvi, p. 407, 1902.

[19] Pick: *Beiträge z. chem. Physiol. u. Path.*, i, p. 351, 1901.

[20] Fuld and Spiro: *Zeitschr. f. physiol. Chem.*, xxxi, p. 133, 1900; Landsteiner: Centralbl. f. Bakt., xxvii, Abt. I, p. 357, 1900; Jacobi: *Beitr. z. chem. Physiol. u. Path.*, I, p. 51, 1901; Cathcart: *Journ. of Physiol.*, xxxi, p. 497, 1904; Glaessner: *Beiträge z. chem. Physiol. u. Path.*, iv, 79, 1904; Simon, Lamar and Bispham: *Journ. of Exper. Med.*, viii, p. 651, 1906; Opie and Barker: ibid., ix, p. 207, 1907.

[21] Gibson and Collins: *Journ. of Biol. Chem.*, iii, p. 233, 1907.

[22] Ledingham (Loc. cit.) also states that the diphtheria antitoxin in goat serum is not invariably linked to the euglobulin fraction as maintained by Pick.

[23] Banzhaf and Gibson: *Loc. cit.*

[24] Ledingham: *Loc. cit.*

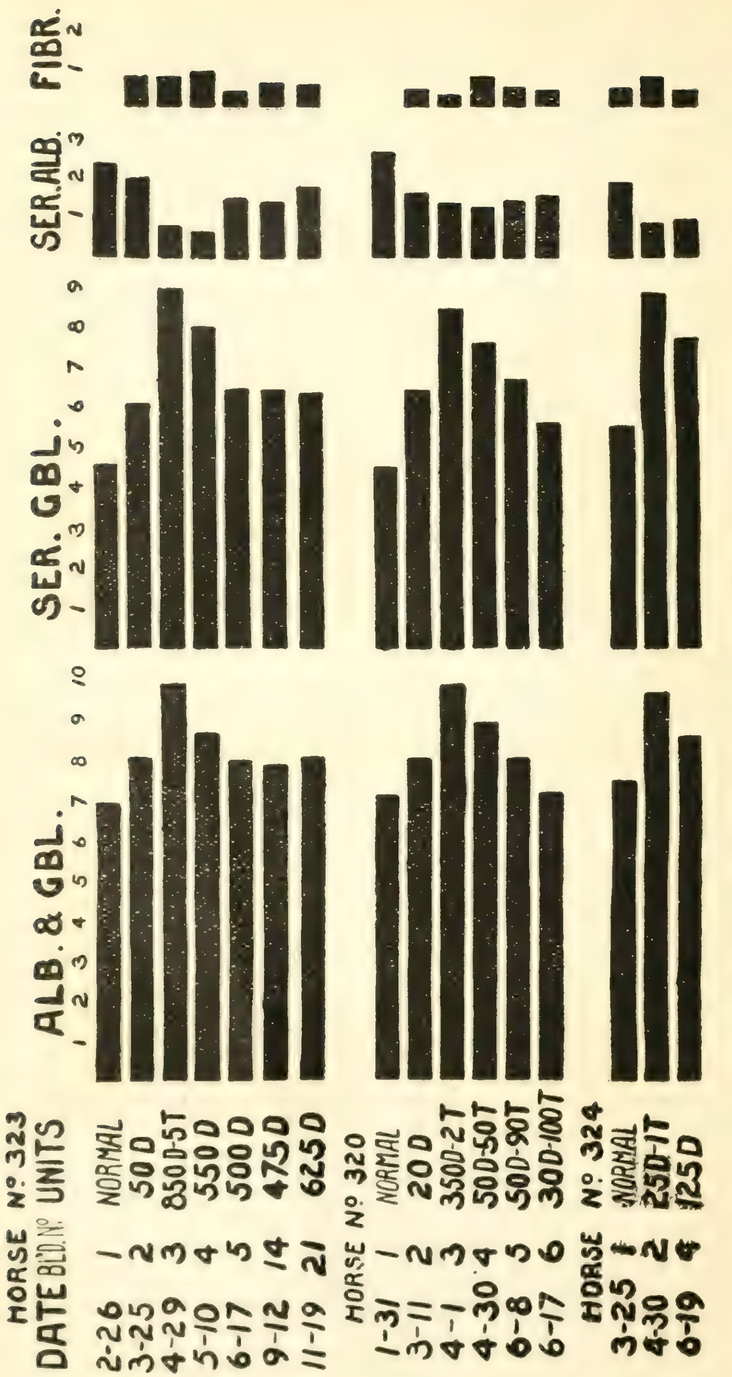
[25] Haslam: *Journ. of Physiol.*, xxxii, p. 267, 1905.

[26] Brieger: *Festschr. f. R. Koch,* Jena, 1903. See also Ledingham: *Loc. cit.*

[27] On horses 318 and 319 there were no intermediate test bleedings.

[28] Moll: *Beiträge z. chem. Physiol. u. Path,* iv, p. 363, 1904.

[29] Dr. K. R. Collins (*Journ. of Exper. Med.*, x, p. 529, 1908) has shown that group and specific bacterial agglutinins can be developed by immunization against yeasts, enzyme preparations and nucleins; these experiments then afford actual proof of the development of widely different anti-substances during immunization. A similar stimulation to production of numerous antibodies in addition to precipitins and specific antitoxins, probably occurs on immunizations with bacterial toxins.


HORSE Nº 323
DATE
BL'D. Nº
UNITS
2-26 1 NORMAL
3-25 2 50 D
4-29 3 850 D-5 T
5-10 4 550 D
6-17 5 500 D
9-12 14 475 D
11-19 21 625 D
HORSE Nº 320
1-31 1 NORMAL
3-11 2 20 D
4-1 3 350 D-2 T
4-30 4 50 D-50 T
6-8 5 50 D-90 T
6-17 6 30 D-100 T
HORSE Nº 324
3-25 1 NORMAL
4-30 2 25 D-1 T
6-19 4 125 D
ALB. & GBL.
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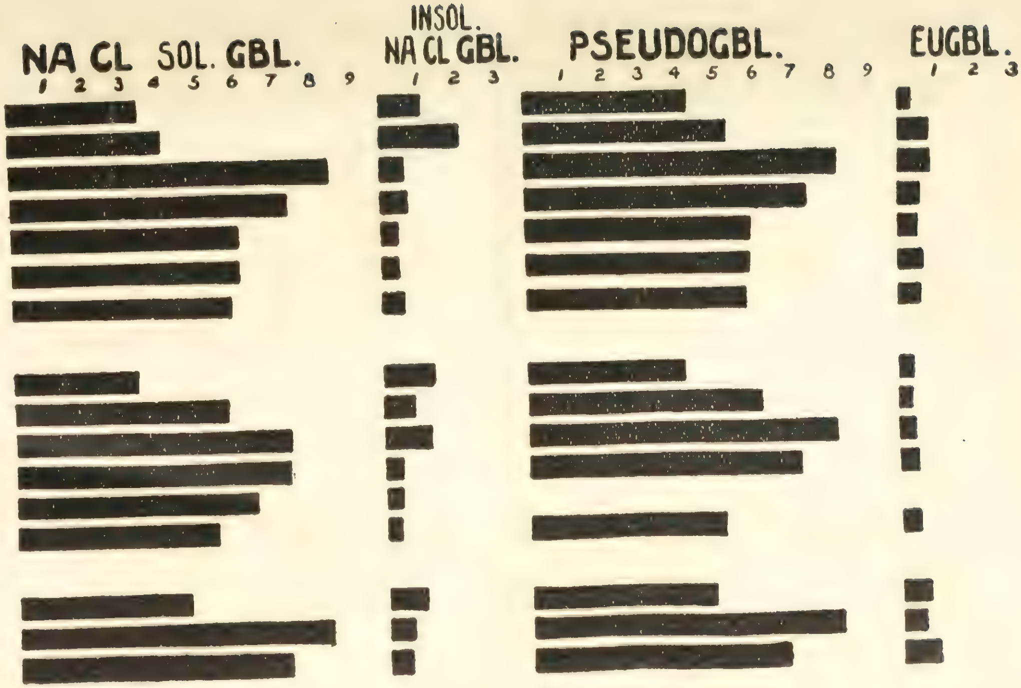
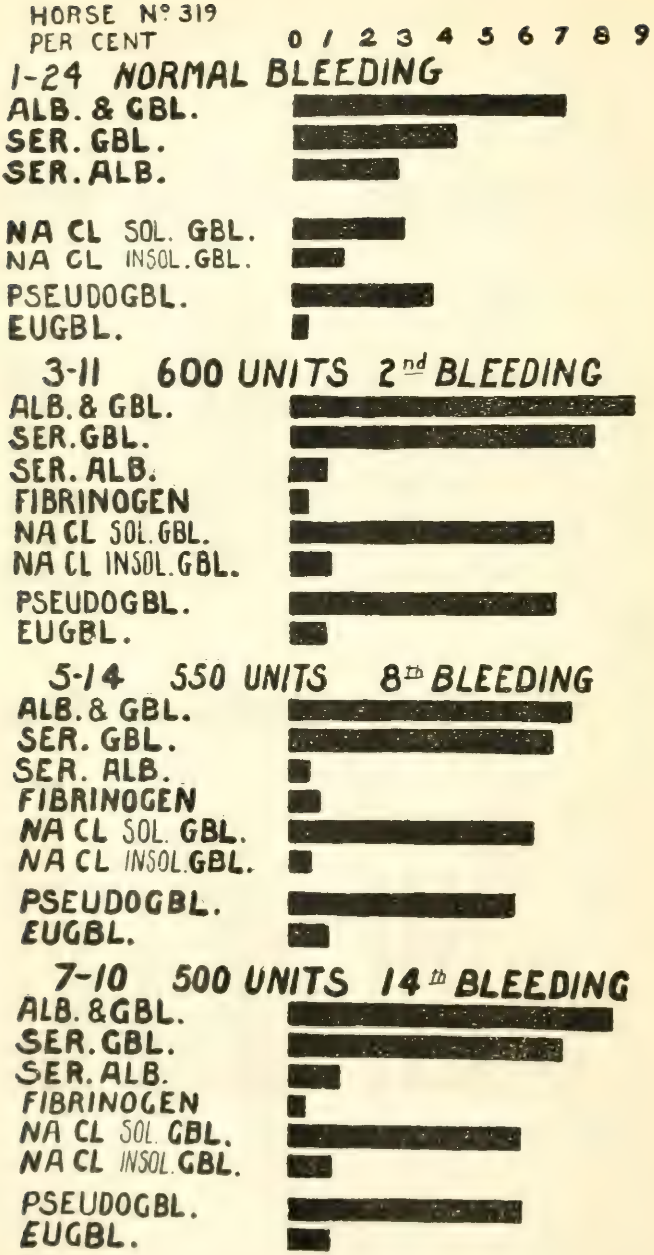
SER. ALB.
1 2 3
FIBR.
1 2



GRAPHIC REPRESENTATION OF PROTEIN DISTRIBUTION.


HORSE No. 319
PER CENT
0 1 2 3 4 5 6 7 8 9
1-24 NORMAL BLEEDING
ALB. & GBL.
SER. GBL.
SER. ALB.
NA CL SOL. GBL.
NA CL INSOL. GBL.
PSEUDOGBL.
EUGBL.
3-11 600 UNITS 2nd BLEEDING
ALB. & GBL.
SER. GBL.
SER. ALB.
FIBRINOGEN
NA CL SOL. GBL.
NA CL INSOL. GBL.
PSEUDOGBL.
EUGBL.
5-14 550 UNITS 8th BLEEDING
ALB. & GBL.
SER. GBL.
SER. ALB.
FIBRINOGEN
NA CL SOL. GBL.
NA CL INSOL. GBL.
PSEUDOGBL.
EUGBL.
7-10 500 UNITS 14th BLEEDING
ALB. & GBL.
SER. GBL.
SER. ALB.
FIBRINOGEN
NA CL SOL. GBL.
NA CL INSOL. GBL.
PSEUDOGBL.
EUGBL.

Horse No. 318

PROTEIN VARIATION DURING IMMUNIZATION*

Date of Bleeding.	Last Amt. Dip. Toxin Inj. About Seven Days Before Date of Bleeding.	Last Amt. Tet. Toxin Inj. About Seven Days Before Date of Bleeding.	Dip. Units per c.c.	Tet. Units per c.c.	Amount Plasma Recovered from Bleeding.	At Dil. 1:10 Total Protein.	At Dil. 1:10 Fibrinogen.	At Dil. 1:10 Serum Albumin.	At Dil. 1:10 Serum Globulin.	At Dil. 1:10 NaCl Insol. Globulin.	At Dil. 1:1.5 Eu Globulin.	At Dil. 1:5 Eu Globulin.	At Dil. 1:10 Eu Globulin.
1-24-07	Normal				400			2.83	4.89	1.68	3.26	1.18	0.65
3-11-07	375	160	600	1	400	9.35	0.48	1.97	6.90	1.44	4.68	1.78	0.98
4- 2-07	475	250	550	10	7300	8.97	0.32	1.95	6.70	0.90	4.84	1.80	0.96
4-10-07	600	375	500	70	7700	8.32	0.42	1.84	6.06	0.45			0.96
4-24-07	Dip.	900	400	110	7850	8.04	0.56	1.47	6.01	0.84	4.31	1.55	0.79
5-14-07	Toxin	1200	200	125	7060	8.89	0.68	0.97	7.24	0.55	4.89	1.80	0.96
5-20-07	Discon-		125	160	6070	8.15	0.53	0.89	6.73	0.69			0.99
6- 8-07	tinued.	1000	50	300	5000	8.18	0.31	1.08	6.79	0.73			0.84
7- 3-07		1200	10	250	6400	7.90	0.27	1.27	6.36	0.70	4.82	1.79	0.96
7-10-07		Tet.	4	200	6300	7.54	0.34	1.25	5.95	0.57			
9- 6-07	400‡	Toxin	200		400	8.11	0.40	1.30	6.41	0.75			0.99
9-25-07	560	Discon-	400		5900	8.20	0.35	1.34	6.51	0.60	4.77	1.75	0.97
10- 9-07	600	tinued.	450		6000	8.19	0.27	1.50	6.42	0.58			1.00
10-19-07	650		500		5900	8.30	0.30	1.66	6.34	0.62			0.96
10-30-07	700		550		6100	8.60	0.21	1.86	6.53	0.59			0.93
11- 8-07	750		550		6300	8.77	0.24	1.88	6.65	0.75			1.00
11-19-07†	750		575		6000	8.72	0.30	1.82	6.60	0.73	4.56	1.68	0.87

* Analyses are given as grams coagulable protein per 100 c.c. of plasma.

† Subsequent bleedings were as follows: 11-27-07, 450 units, 6,100 c.c.; 12-6-07, 450 units, 5,850 c.c.; 12-16-07, 450 units, 5,800 c.c.; 12-27-07, 400 units, 6,200 c.c.; 1-6-08, 350 units, 6,200 c.c., and 1-7-08, 200 units, 8,000 c.c. The horse died while bleeding on 1-7-08.

‡ Diphtheria toxin injections resumed August 14, 1907.

PROTEIN VARIATION DURING IMMUNIZATION*

Horse No. 323

Date of Bleeding.	Last Amt. Dip. Toxin Inj. About Seven Days Before Date of Bleeding.	Last Amt. Tet. Toxin Inj. About Seven Days Before Date of Bleeding.	Dip. Units per c.c.	Tet. Units per c.c.	Amount Plasma Recovered from Bleeding.	At. Dil. 1:10 Total Protein.	At Dil. 1:10 Fibrinogen.	At Dil. 1:10 Serum Albumin.	At Dil. 1:10 Serum Globulin.	At Dil. 1:10 NaCl Insol. Globulin.	At Dil. 1:1.5 Eu Globulin.	At Dil. 1:5 Eu Globulin.	At Dil. 1:10 Eu Globulin.
2-16-07	Normal				400			2.33	4.60	1.16	2.21	0.68	0.37
3-25-07	110	70	50		400	8.79	0.74	1.98	6.07	2.06	4.06	1.45	0.82
4-29-07	400	525	850	5	400	10.49	0.71	0.78	9.00	0.66	4.50	1.53	0.85
5-10-07		Tet.	550		6000	9.47	0.80	0.66	8.01	0.70	3.29	1.22	0.67
6-17-07	375	Toxin	500		7700	8.29	0.39	1.45	6.45	0.46	2.85	1.00	0.57
7- 2-07	475	Discon-	500		8000	8.36	0.29	1.33	6.74	0.64			
7-10-07	500	tinued.	450		6200	8.16	0.50	1.25	6.41	0.61	3.99	1.39	0.75
7-18-07	550		450		6200	7.92	0.32	1.14	6.46	0.79	3.80	1.30	0.71
7-29-07	600		450		6200	8.08	0.48	1.24	6.36	0.41			0.73
8- 9-07	650		425		6100	8.00	0.40	1.30	6.30	0.51			0.69
8-17-07	700		425		6200	8.20	0.47	1.32	6.41	0.58			0.68
8-27-07	750		425		6300	8.15	0.44	1.25	6.46	0.46			0.70
9- 3-07			425		6000	8.30	0.50	1.30	6.50	0.50			0.67
9-12-07	800		475		6250	8.35	0.55	1.33	6.47	0.47			0.68
9-19-07	800		500		5200	8.45	0.46	1.40	6.59	0.52			0.70
9-28-07	800		550		6400	8.30	0.50	1.55	6.25	0.50			0.65
10- 9-07	800		575		6100	8.50	0.45	1.75	6.20	0.55			0.55
10-19-07	800		550		6200	8.65	0.50	2.00	6.15	0.53			0.57
10-30-07	850		625		6200	8.71	0.53	2.18	6.00	0.48	2.87	0.99	0.55
11- 8-07	850		600		6100	9.10	0.53	2.00	6.57	0.64	2.92	1.01	0.55
11-19-07†	850		625		6300	8.56	0.51	1.73	6.32	0.57	3.15	1.12	0.61

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* Analyses are given as grams coagulate protein per 100 c.c. plasma.

† Subsequent bleedings were as follows: 11-27-07, 600 units, 5,900 c.c.; 12-6-07, 600 units, 5,800 c.c.; 12-16-07, 600 units, 5,900 c.c.; 12-27-07, 500 units, 5,900 c.c.; 1-6-08, 400 units, 7,700 c.c.; 1-14-08, 350 units, 7,500 c.c.; 1-22-08, 400 units, 8,000 c.c.; 1-31-08, 400 units, 7,800 c.c.; 2-7-08, 425 units, 8,000 c.c.; 2-15-08, 425 units, 8,000 c.c.; 2-24-08, 475 units, 8,000 c.c. 3-4-08, 300 units, 8,000 c.c.; 3-12-08, 300 units, 8,000 c.c. There were no further bleedings. Over 200 liters of undiluted plasma were thus obtained from this one horse, representing the actual withdrawal of at least 275 liters of whole blood. The horse died suddenly on 7-30-08; toxin had not been given after 3-12-08.

PROTEIN VARIATION DURING IMMUNIZATION*

Horse No. 319

Date of Bleeding.	Last Amt. Dip. Toxin Inj. About Seven Days Before Date of Bleeding.	Last Amt. Tet. Toxin Inj. About Seven Days Before Date of Bleeding.	Dip. Units per c.c.	Tet. Units per c.c.	Amount Plasma Recovered from Bleeding.	At. Dil. 1:10 Total Protein.	At Dil. 1:10 Fibrinogen.	At Dil. 1:10 Serum Albumin.	At Dil. 1:10 Serum Globulin.	At Dil. 1:10 NaCl Insol. Globulin.	At Dil. 1:1.5 Eu Globulin.	At Dil. 1:5 Eu Globulin.	At Dil. 1:10 Eu Globulin.
1–24–07	Normal				400			2.80	4.24	1.29	2.43	0.79	0.48
3–11–07	375	160	600	1	400	9.32	0.43	0.99	7.90	1.10	5.39	1.78	0.98
4– 2–07	475	250	550	2	7100	8.55	0.33	1.17	7.05	0.82			1.00
4–10–07	600	375	525	4	8000	8.51	0.43	1.25	6.83	0.50	5.15	1.89	1.00
4–18–07	650	Tet.	550		7850	7.85	0.52	0.87	6.46	0.69			0.88
4–27–07	700	Toxin	575		5600	8.66	0.71	0.89	7.06	0.69	5.75	2.00	1.10
5– 7–07	700	Discon-	550		8000	8.02	0.61	0.81	6.60	0.49			0.90
5–14–07	750	tinued.	550		8700	8.20	0.82	0.52	6.86	0.57			0.98
5–23–07	750		550		7900	8.66	0.74	0.55	7.37	0.67			0.99
6– 1–07	850		550		6400	8.86	0.76	0.75	7.35	0.56			1.02
6–10–07	850		550		6250	8.70	0.58	0.67	7.45	0.98			0.98
6–17–07	900		525		5950	8.44	0.56	0.90	6.98	0.88			0.89
6–27–07	900		525		5700	8.65	0.65	1.21	6.79	1.00			0.98
7–10–07	900		500		5900	8.82	0.41	1.30	7.11	1.05			1.00
Horse No. 320													
1–31–07	Normal				400			2.60	4.52	1.30	2.03	0.72	0.40
3–11–07	260	100	20		400	8.50	0.47	1.57	6.46	0.82	1.80	0.60	0.34
4– 1–07	500	350	35	2	400	10.14	0.34	1.32	8.48	1.20	2.12	0.74	0.41
4–30–07	600	1050	50	50	400	9.63	0.74	1.22	7.67	0.45	2.61	0.90	0.50
6– 8–07	Dip.	1350	50	90	5600	8.55	0.49	1.35	6.71	0.40			
6–17–07	Toxin Discon-	1100	30	100	7100	7.64	0.41	1.59	5.64	0.35	2.58	0.90	0.49
6–26–07	tinued.	1100	10	90	6200	7.70	0.48	1.61	5.61	0.27	2.69	0.93	0.51

* Analyses are given as grams coagulate protein per 100 c.c. of plasma.

PROTEIN VARIATION DURING IMMUNIZATION *

Date of Bleeding.	Last Amt. Dip. Toxin Inj. About Seven Days Before Date of Bleeding.	Last Amt. Tet. Toxin Inj. About Seven Days Before Date of Bleeding.	Dip. Units per c.c.	Tet. Units per c.c.	Amount Plasma Recovered from Bleeding.	At Dil. 1:10 Total Protein.	At Dil. 1:10 Fibrinogen.	At Dil. 1:10 Serum Albumin.	At Dil. 1:10 Serum Globulin.	At Dil. 1:10 NaCl Insol. Globulin.	At Dil. 1:1.5 Eu Globulin.	At Dil. 1:5 Eu Globulin.	At Dil. 1:10 Eu Globulin
Horse No. 324**													
3-25-07	100	70	1/4		400	7.88	0.45	1.88	5.55	0.98	3.72	1.37	0.74
4-30-07	400	525	25	1	400	10.43	0.76	0.83	8.84	0.63	3.30	1.18	0.65
5-31-07	725	Tet. Toxin Discontinued.	125		400	9.49	0.61	0.64	8.24	0.56			0.84
6-19-07	900		125		8000	9.06	0.42	0.93	7.71	0.53	4.79	1.75	0.98
Horse No. 325													
2-26-07	Normal				400			2.61	3.68	0.91	1.79	0.76	0.42
3-25-07	110	70	4		400	7.78	0.50	2.25	5.03	1.54	3.78	1.39	0.76
4-30-07	400	525	300	4	400	8.65	0.59	1.16	6.90	0.49	5.15	1.84	1.00
5-29-09		1000	200	25	400	7.74	0.54	1.41	5.79	0.50	4.72	1.74	0.98
7- 2-07	550	Tet. Toxin Discontinued.	150		6800	8.11	0.39	1.63	6.09	0.90			1.00
7-10-07	600		150		5300	8.45	0.35	2.31	5.79	0.82			0.99
9- 3-07	1050		200		7300	7.95	0.40	2.10	5.45	0.90			0.97
9-17-07	1200		250		6100	8.10	0.41	2.30	5.39	0.84			0.95
9-28-07	1200		200		5350	7.90	0.36	2.29	5.25	0.80			0.93
10- 9-07	1200		200		5400	8.00	0.38	2.35	5.27	0.70			0.80
10-19-07	1300		175		5600	7.80	0.40	2.41	4.99	0.65			0.85
10-30-07	1300		150		1100	7.86	0.36	2.44	5.06	0.58	4.20	1.54	0.81
11- 8-07			125		400	7.61	0.30	2.55	4.76	0.64			0.67
11-19-07			75		400	6.95	0.55	2.29	4.11	0.37	2.37	0.81	0.52
Horse No. 326													
3- 6-07	Normal				400	7.15	0.33	2.82	4.00	1.09	2.06	0.77	0.42
4- 1-07	100	50	5		400	8.47	0.46	2.38	5.63	0.96	3.52	1.26	0.70
4-30-07	350	400	600	1	8000	9.41	0.64	1.16	7.61	0.42	4.68	1.66	0.90
5-10-07	400	Tet. Toxin Discontinued.	600		8000	8.08	0.72	1.44	5.92	0.63	3.87	1.34	0.72
5-18-07	450		600		7800	8.73	0.79	0.98	6.96	0.61	3.78	1.29	0.70
5-29-07	500		600		8000	8.73	0.74	0.97	7.02	0.74	3.93	1.38	0.72
6-13-07	500		550		5600	10.52	0.68	0.61	9.23	1.01	6.46	2.38	1.30

* Analyses are given as grams coagulable protein per 100 c.c. of plasma.

THE FURTHER SEPARATION OF ANTITOXIN FROM ITS ASSOCIATED PROTEINS IN HORSE SERUM

By Edwin J. Banzhaf

The literature concerning means of purification of anti-bodies and their chemical characteristics has been thoroughly reviewed by Gibson,[1] Ledingham,[2] Banzhaf and Gibson,[3] and Brieger and Kraus.[4]

Stark[5] was the first to report that by heating for one hour at 56° C., ovalbumin could be converted into a body, which, because of its precipitation and solution reactions, and its composition, was obviously a globulin. Later Noll[6] showed the same to be true of albumin in rabbit, dog and horse serum.

My experiments were to ascertain the resulting conditions after heating antitoxic horse serum, citrated plasma and Gibson's concentrated and partially purified antitoxic globulin solution.

Heating an antitoxic serum prepared by the Gibson method[1] caused an elimination of 23 per cent. protein and an increase of antitoxic units per gram protein of 30 per cent. over the native serum. A number of specimens of the same antitoxic serum were heated for from 6 to 72 hours in closed containers, at a temperature of 57° C. After cooling to room temperature, the sera were saturated with sodium chloride and brought up to a dilution of 1 : 10 with saturated sodium chloride solution. Twelve hours later the resulting precipitations were filtered off. Potency tests on these filtrates showed a loss of 5 per cent. after heating 6 hours, and an increasing loss up to 22 per cent. after heating 72 hours. The protein converted into an insoluble condition (in saturated sodium chloride solution) was 30 per cent. for the 6-hour period, increasing up to 48 per cent. for the 72-hour period. The increase of antitoxic units, per gram protein, was 35 per cent. after 6 hours' heating, increasing up to 53 per cent. after 48 hours.

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[1] *Journal of Biolog. Chem.*, i, p. 161, 1906.
[2] *Journal of Hyg.*, vii, p. 65, 1907.
[3] *Journal of Biolog. Chem.*, iii, p. 253, 1907.
[4] *Berl. klin. Woch.*, xliv, p. 946, 1907.
[5] *Zeitschr. f. Biol.*, xl, p. 494, 1900 (new series, vol. 22).
[6] *Hofmeister's Beiträge*, iv, p. 563, 1904.

Owing to the larger per cent. destruction of antitoxin at the 72-hour heating than the per cent. increase converted, the potency per gram protein represented only 52 per cent. increase over the native serum. On separating the remaining unconverted albumin from this series, the increase of antitoxic units, per gram protein, was 60 per cent. after 6 hours' heating, increasing to 78 per cent. after 48 hours. The 72-hour heating showed an increase of 73 per cent. over the native serum.

Citrated plasma under the same conditions gave practically the same results. Gibson's antitoxic globulin solution (blood alkalinity) containing only that globulin soluble in saturated sodium chloride solution was heated under the same conditions. The potency loss was 5 per cent. for the 6-hour period, and an increasing loss up to 23 per cent. for the 72-hour period. The soluble globulin converted into an insoluble condition (in saturated sodium chloride solution) was 30 per cent. after 6 hours' heating, increasing to 47 per cent. after 72 hours. The increase of antitoxic units, per gram of protein, was 37 per cent. after 6 hours' heating, increasing to 54 per cent. for the 24 hours. Here again the 72-hour heating period caused a larger per cent. destruction of antitoxin than per cent. globulin converted into an insoluble condition (in saturated sodium chloride solution), representing only an increase of 46 per cent., per gram protein, over Gibson's antitoxic globulin solution.

This work is being continued, for it is believed to be practically and scientifically important, and may throw some light on the chemical characteristics and the nature of antitoxins.

TABLE I

HORSE 359 CITRATED PLASMA 475 UNITS PER C.C.

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein Minus Fibrinogen.	% Coagulable Albumin.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted into Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Coagulable Protein Soluble in NaCl Sol. minus Albumin.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxi on Heating.
		7.03				6,756			6,756		
		7.03	.97	6.36		7,468	10.53	5.39	8,812	30.43	
6	57°	7.03	.77	4.97	21.85	9,054	34.01	4.20	10,714	58.58	5.26
8	57°	7.03	.69	4.81	24.37	9,355	38.46	4.12	10,922	61.67	5.26
15	57°	7.03	.67	4.64	27.04	9,698	43.54	3.97	11,335	67.77	5.26
18	57°	7.03	.65	4.46	29.87	9,865	46.01	3.81	11,548	70.92	7.36
24	57°	7.03	.61	4.09	35.69	10,391	53.80	3.48	12,212	80.75	10.52
48	57°	7.03	.50	3.63	42.92	10,743	59.01	3.13	12,460	84.42	17.89
72	57°	7.03	.37	3.32	47.80	10,542	56.03	2.95	11,864	75.60	26.31

TABLE II

HORSE 353 SERUM. 775 UNITS PER C.C.

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein.	% Coagulable Albumin.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted into Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Coagulable Protein Soluble in Sat. NaCl Sol. minus Albumin.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxin on Heating.
		7·39				10,487			10,487		
		7·39	1.50	7·20		10,763	2.63	5·70	13,596	29.64	
6	57°	7·39	.81	5·19	27·91	14,161	35·03	4·38	16,780	60.00	5·16
8	57°	7·39	·75	5·02	30·27	14,641	39·61	4·27	17,214	64.14	5·16
15	57°	7·39	·74	4.82	33·05	15,041	43·42	4·08	17,769	69.43	6.45
18	57°	7·39	·73	4·73	34·30	15,327	46.15	4·00	18,125	72.83	6.45
24	57°	7·39	.64	4·44	38·33	15,765	50·32	3·80	18,412	75·56	9.67
48	57°	7·39	.56	4·05	43·75	16,049	53·03	3·49	18,624	77·59	16.12
72	57°	7·39	·45	3·76	47·77	15,960	52.18	3·35	18,126	72.84	22.58

TABLE III

PREP. 138 DILUTED WITH EQUAL VOLUME WATER. EACH C.C.-1100 UNITS

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted to Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxin on Heating.
		8.70		12,643		
6	57°	6.05	30.45	17,355	37.26	4.54
8	57°	5.94	31.72	17,676	39.80	4.54
15	57°	5.72	34.26	17,919	41.73	6.81
18	57°	5.55	36.20	18,018	42.51	9.09
24	57°	5.40	37.93	18,518	46.46	9.09
48	57°	4.76	45.28	19,432	53.70	15.90
72	56–57°	4.60	47.12	18,478	46.15	22.72

TABLE IV

PREP. 135 DILUTED WITH EQUAL VOLUME WATER. EACH C.C.-475 UNITS

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted to Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxin on Heating.
		6.78		7,005		
6	57°	4.88	28.02	9,221	31.63	5.26
8	57°	4.80	29.20	9,375	33.83	5.26
15	57°	4.60	32.15	9,782	39.74	5.26
18	57°	4.40	35.10	9,886	41.12	8.42
24	57°	4.30	36.58	10,116	44.41	8.42
48	57°	3.85	43.21	10,389	48.30	15.78
72	57°	3.53	47.93	10,198	45.58	24.21

TABLE V

PREP. 130 DILUTED 750 UNITS PER C.C.

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted to Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxin on Heating.
		10.70		7,009		
6	58°	7.14	33.27	9,803	39.86	6.66
8	58°	7.09	33.73	9,873	40.86	6.66
10	58°	7.06	34.01	9,915	41.46	6.66
12	58°	6.94	35.14	10,086	43.90	6.66
14	58°	6.59	38.41	4	46.12	10.00
16	58°	6.49	39.34	10,400	48.38	10.00
18	58°	6.35	40.65	10,629	51.64	10.00
24	58°	6.08	43.17	10,690	52.50	13.33

THE ROUTINE PROCESS FOR THE FURTHER PURIFICATION OF THE ANTITOXIC PROTEINS IN HORSE SERUM FOR THERAPEUTIC USE

By Edwin J. Banzhaf

The Gibson[1] process of purification and concentration was based on the knowledge that the antitoxin is associated only with those globulins which are soluble in saturated sodium chloride solution. In the average normal horse serum the proteins are distributed as follows: Albumin, 40 per cent.; globulin soluble in saturated sodium chloride solution, 42 per cent.; globulin insoluble in saturated sodium chloride solution, 18 per cent. From this it would seem that about 58 per cent. of the total proteins was eliminated and that a considerable purification of the serum had been effected.

Investigation carried on by Banzhaf and Gibson[2] showed that the proteins of immunized horses fluctuated considerably. These authors estimated quantitatively the albumins, the pseudoglobulins and euglobulins at various dilutions in eleven normal horses and followed the protein distribution of these horses every week through their entire immunization. Their work showed that the total proteins of the immunized horses were increased, and that this increase was at its highest (20 to 45 per cent.) after about two months' treatment. This was true of all the horses, regardless of the unit content. They also showed that the non-antitoxic proteins, the albumin and euglobulin were greatly diminished, while the pseudoglobulin carrying the antitoxin was greatly increased.

In the average antitoxic horse under treatment about three months the proteins of the serum are distributed as follows: albumin, 12 per cent.; globulin carrying the antitoxin, 78 per cent.; globulin insoluble in saturated sodium chloride solution, 10 per cent. Thus it can be seen that the Gibson process will only eliminate about 22 per cent. non-antitoxic proteins.

Early in the year I experimented with antitoxic sera heated at different temperatures and periods of time and found changes taking place in the proteins which allowed me to further purify the antitoxin. Thus, if I heated antitoxin sera of 600 units per c.c. containing albumin 12 per cent., globulin carrying the antitoxin 78 per cent., globulin non-antitoxic 10 per cent., for from 12 to 15 hours at a time at a temperature of 57° C., a rearrangement of the precipitating characteristics of the proteins resulted; so that now there would be albumin 9 per cent., globulin and all the antitoxin 50 per cent., and globulin non-antitoxic 41 per cent., showing an elimination of 50 per cent. non-antitoxic proteins. The loss of antitoxin on heating at a temperature of 57° C. for from 12 to 15 hours was from 5 to 7 per cent.

The following routine process is based on the results recorded in the tables following the preceding abstract "On the Further Separation of Antitoxin from Its Associated Proteins in Horse Serum." This process has given very satisfactory results and is similar to the original process first outlined by Gibson[1] in this laboratory and later improved by Banzhaf and Gibson.[2]

For routine work in lots 50 or 60 liters, I do not concentrate more than five or six times in order to keep the total proteins in the finished product below 20 per cent.

Antitoxin plasma, in lots of 50 liters, is heated in a water bath at a temperature of 57° C. for from 12 to 15 hours, cooled to room temperature and diluted with 25 liters of water. Saturated ammonium sulphate is added in the proportion: 3 c.c. saturated ammonium sulphate in 10 c.c. diluted plasma. The resulting fractional precipitate will contain all the euglobulin together with a small amount of antitoxin and pseudoglobulin. This precipitate is taken up in saturated sodium chloride solution and carried on according to Gibson's original process. The filtrate from the above precipitate is measured and brought up to 54 saturation ammonium sulphate, according to the following proportions: 2.4 c.c. saturated ammonium sulphate in 10 c.c. filtrate. The resulting precipitate contains only those globulins soluble in saturated sodium chloride solution together with the antitoxin. The second precipitate is pressed to remove excess of ammonium sulphate solution containing the albumin and placed in dialyzing bags. Dialysis is continued until all traces of ammonium sulphate are removed. This requires six to eight days' dialysis in running water. After dialysis is complete, sufficient sodium chloride is added to bring the solution up to 0.08 per cent. The first precipitate, even after carrying it through the Gibson process, will also require six to eight days' dialysis to remove traces of ammonium sulphate.

In our former methods on fractioning and separating, the second precipitate was dissolved in saturated sodium chloride and the proteins containing the antitoxin precipitated from this menstruum with acetic acid. Our reason for doing this was to get rid of as much of the ammonium sulphate as possible before dialysis. Now, however, with greatly improved methods of pressing the precipitate, it can be dialyzed directly, thereby saving the otherwise necessary loss of time, antitoxin and other expenses.

The concentration of antitoxin effected with the method outlined is from five to eight times, depending on the quantity and distribution of the protein in the antitoxic plasma. The loss of antitoxin with this method ranges from 16 to 22 per cent.

Rashes of the urticarial character with little or no accompanying constitutional disturbances are less frequent after the therapeutic administration of this further purified product than with the former products.

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[1] Journal of Biolog. Chem. 1, p. 161, 1906, and Collected Studies from the Research Laboratory, Vol. I, 1905.

[2] Journal of Biolog. Chem., III, p. 253, 1907, and Collected Studies from Research Laboratory, Vol. III, 1907.

THE PRODUCTION OF DIPHTHERIA ANTITOXIN DURING THE YEAR 1908

By Edwin J. Banzhaf

The total amount of antidiphtheritic citrated plasma produced in the laboratory during the year 1908 was 1,584,000 c.c. This amount was recovered from 220 bleedings from 24 horses. The highest potency was about 800 units per c.c.; the lowest about 100 units per c.c.

One million three hundred and eighty-five thousand three hundred c.c. of antitoxic citrated plasma, with an average potency of 320 units per c.c. were refined and concentrated by a fractional method devised in this laboratory.

From this amount were obtained 370,940 c.c. of antitoxic globulin solution, with an average potency of 1,009 units per c.c. The highest potency was 2,100 units and the lowest 600 units per c.c.

The following table shows the production of antitoxin from the individual horses:

Horse No.	Number of Bleedings in 1908.	Citrated Plasma in c.c. Obtained in 1908.	Highest Potency in Units per c.c.	Average Potency in Units per c.c.
318	2	14,200	350	275
323	16	122,400	475	358
333	7	50,800	300	221
334	13	92,350	375	309
335	3	22,250	250	208
336	12	94,000	350	279
338	17	123,470	350	289
340	2	15,500	200	200
342	17	126,100	400	318
343	9	59,700	400	300
344	3	23,100	175	158
345	1	18,000	250	250
346	8	55,100	400	343
347	5	34,100	300	230
348	2	13,700	200	200
349	5	37,700	300	250
350	11	81,200	400	309
351	12	95,850	375	341
352	12	89,000	450	352
353	29	195,470	800	473
354	3	19,350	350	313
355	5	34,450	300	245
356	3	21,500	300	213
359	23	144,710	500	431
24	220	1,584,000		

ROUTINE DIAGNOSIS OF RABIES FOR THE YEAR 1908

Anna W. Williams

The following is a comparative table of the number of animals sent to the laboratory for diagnosis during 1908 and the previous two years:

Month.	1906.		1907.		1908.	
	Positive.	Negative.	Positive.	Negative.	Positive.	Negative.
January...	7	1	18	3	12	7
February..	5	1	12	6	20	3
March....	9	1	12	2	25	8
April......	5	1	9	5	23	6
May......	12	1	13	3	20	18
June......	10	4	11	7	22	21
July	3	7	10	5	9	26
August....	9	5	19	6	16	33
September.	7	2	21	8	8	21
October....	9	8	14	5	9	7
November.	11	1	24	4	7	11
December.	14	5	18	5	4	9
Total.....	101	37	181	59	175	170

It will be seen that there is a decided drop in the number of rabies cases during the latter part of 1908. This is no doubt due to the active destruction of stray dogs by the Health Department at the beginning of this time.

The technic of our diagnostic work remained the same as that of the previous year, as published in Volume III of these studies.

REPORT ON THE AGGLUTINATION TEST FOR GLANDERS

By Marie Grund, M.D.

During the six months from October, 1908, to March, 1909, the sera of 979 horses have been tested at this laboratory for the agglutination reaction with the *Bacillus mallei.*

The results of these tests are as follows:

TABLE I

Dilution in which the sera agglutinated

Dilution.	1–200 or Less.	1–500	1–1000	1–2000	1–5000	1–10,000
No. of Sera..	379	224	196	102	46	32

Eight hundred and nineteen of the sera came from horses in three stables, and of these a detailed report on clinical symptoms, mallein reaction and final disposition has not yet been received. It may be mentioned, however. that out of a total of 390 horses in one of these stables, 150 have been destroyed on clinical evidence. This accords, roughly, with the result of the laboratory test which shows that 43.6 per cent. of the 390 sera agglutinated the *Bacillus mallei* in dilutions of 1-1000 and over.

Of the remaining 160 horses, for which clinical data could be obtained, only two were reported as actually diseased on the slip accompanying the serum; according to the final report, however, 51 showed symptoms of glanders. In four of these which came to autopsy the clinical diagnosis was verified by the bacteriological findings.

Table II shows the agglutination reaction grouped with relation to the clinical symptoms, and, as far as could be ascertained, to the mallein reaction. In quite a number of cases mallein was not used, the diagnosis being based on clinical symptoms and the agglutination test.

In the main these results agree with those obtained in previous years. While with higher dilutions the percentage of error diminishes, it is interesting to note that in two apparently normal cases, in which the serum agglutinated the *Bacillus mallei* in dilutions of 1/10,000, the mallein test bore out the clinical diagnosis against the laboratory test. The fact that less than 50% of the horses whose serum agglutinated the specific organism in high dilution (2,000 and 5,000) were clinically glandered, while in the doubtful cases the mallein test was negative, also would indicate that too much dependence must not be put upon the agglutination reaction as an absolutely diagnostic measure but that it must merely be considered as corroborating the other means of diagnosis.

TABLE II

Dilution.	No. Sera.	Clinical Sympt. of Glanders.	Mallein.		Not Used.	Working.	Destroyed.
200 or less	56	8	1	12	43	*43	9
500	28	7	1	13	14	20	8
1,000	31	13	5	9	17	18	13
2,000	19	8	5	7	7	10	9
5,000	13	5	2	7	4	8	5
10.000	13	11	3	2	8	2	11
Total	160	52	17	50	93	101	55

* Four horses under observation to be re-examined.

It may be of interest to state that in addition to the sera from horses, specimens of serum from two cases of glanders in the human subject have been tested, both of which reacted promptly in a dilution of 1/5000.

STUDIES ON INTESTINAL AMEBAS AND ALLIED FORMS

BY

ANNA W. WILLIAMS, M.D., AND CAROLINE R. GURLEY

The study of the intestinal amebas was undertaken by us for the following reasons:

First, because there had been so little minute work done on the life cycle of these forms that the question as to whether a distinct species causes amebic dysentery was unsettled; and, second, because of an apparent resemblance in morphology between certain stages in the life history of amebida and of the vaccine and Negri bodies, it was thought that a better knowledge of the former might throw more light upon the nature of the latter.

Critical Review of Recent Work—The two most recent articles on this subject, one by Walker and the other by Craig, contain almost complete bibliographies and reviews of work done, so it is only necessary for us to review the latest reports.

The majority of studies on the intestinal amebas before the work of Musgrave and Clegg were made on smears, hanging drops, and sections, taken directly from the intestinal contents and from the infected tissues of human beings and of inoculated kittens and puppies. There had been a few more or less unsuccessful attempts made to cultivate these micro-organisms on artificial media, but no extensive culture work was reported until 1904 when the studies of Musgrave and Clegg appeared.

These authors, however, did not describe the minute morphologic changes of their organisms; so, up to the time we began to do our work, Schaudinn was the only one who had attempted to give anything like a full life cycle of these forms. Since then his work has been partially corroborated by Prowazek, Craig and Wenyon.

Walker's article appeared in February, 1908. It contains a pretty complete historical review, but his original studies are somewhat disappointing to us, owing to the fact that they were studies only of the artificial cultures, and not of the organisms in their natural habitat. And though he states that "caution must be exercised in the construction of a life cycle from development on artificial media, since it is possible that not all stages may occur under these conditions," he proceeds, without making control observations on the organisms in their natural habitat, to construct a life cycle and to describe eight new species from the forty-four cultures he has studied. He states that he has made this classification after a critical examination of the characteristics of the ameboid, the encysted, and the sporulating stages as they occur in cultures, but nowhere does he state definitely what these characteristics are. He has presumably given them in his descriptions of the different species at the end of his article, but on tabulating his eight new species and the one he obtained from the Philippines, according to the characteristics he gives, we can find only differences noted which seem to us to be too slight and variable for use in the determining of new species.

In regard to the technic of his culture work, Walker claims to have devised a new method of study, which he calls "the hanging plate method," which he praises highly, suggesting that it might be used with advantage by bacteriologists. This method *has* been used by bacteriologists for many years under the name of the "hanging mass" method.

Walker's culture work on the whole, that is, the preparation of the media, the isolation of pure cultures, the effects of oxygen, moisture and temperature, is practically only a corroboration of the work of Musgrave and Clegg, published in 1904. We have already found that a number of their statements in regard to media and conditions of growth are not true, at least for the organisms with which we are working. For instance, they state that the culture medium must be slightly alkaline, 1 per cent. to phenolphthalein. All of our cultures grow abundantly on a medium that is 1.5 per cent. *acid* to this indicator. This medium, moreover, is the ordinary nutrient agar, and all previous workers, including Walker, have said that this nutrient medium is not fitted for the growth of amebae because it allows too rich a growth of bacteria. They, therefore, recommend a *special* agar medium containing only a small amount of nutriment. We have found, however, that all of our organisms grow better in the long run on the nutrient agar than on the special medium.

We have also found, contrary to these other observations, that growth is rapid and abundant at blood heat, if the right kinds of bacteria (those that do not grow rapidly at this temperature) are used as food.

One new point which Walker has given in his technic is designed to isolate a single ameba and obtain a pure growth from it. A fresh agar plate is streaked with material from a previous culture, then covered and inverted on the stage of a microscope. With low magnification the location of a single ameba at the edge of the streak is determined, and its position marked with ink or wax pencil on the bottom of the Petri plate with a V-shaped mark, the apex of which just enclosed the ameba. The plate is then turned right side up, the cover removed, and the V-shaped mark traced with carbolic vaseline on the surface of the agar. This isolates the one organism from the others, as no growth can take place through the carbolic vaseline. We have gotten a method far simpler than this, which will be described below.

The morphologic studies of Walker are chiefly on the living organism. He confesses that the technic of his fixed and stained preparations may be faulty, and that, therefore, his observations of minute changes may possibly be incorrect. He says that in all of his forty-seven cultures reproduction takes place in only two ways; first, by simple binary division, and, second, by what he calls sporulation. In the former case he describes briefly amitotic division in the living ameboid forms, and calls attention to the fact that no other observer has reported anything but amitotic division during the vegetative stage. Schaudinn describes for his *Entameba coli* and autogamous *sexual* process occurring in the *resting* stage (spore stage) in which the nuclei divide by a primitive mitosis, but no other author, except those corroborating his work, has reported mitotic division in the human parasitic forms. Wenyon has described mitosis in the vegetative stage of an ameba from the intestines of the mouse. Walker states that in one case he found in a specimen from a culture of *"ameba coli"* what appeared to be an anaphase of a mitotic division. Therefore, he thinks it is possible that some forms at certain stages may divide mitotically.

In regard to his reproduction by sporulation, which, as he describes it, is new, his descriptions are again indefinite. He does not define the term sporulation as he uses it, but since he says he has been unable to discover any indications of a sexual process, his spore formation probably corresponds either with the process of schizogony or with that of simple budding; and yet he compares this process with the extrusion of spores described by Schaudinn as occurring in *Entameba histolytica* and which Schaudinn considers part of a sexual process. Walker is of the opinion, however, that if a sexual process exists in this group, it probably takes place "by the interchange of reproductive nuclear matter, or chromidia," which he says functions in sporulation.

The study of the process of encystment in these amebas Walker considers a very important aid in the differentiation of species. The size of the cysts, their contents, and the structure of their walls are all dwelt upon, but, as I have already stated, no marked differences are brought out. The variation in size of a single species is great; the contents are spoken of as coarsely or finely granular and the walls as single or double, scalloped or entire, but he does not state whether these appearances vary with the condition of the medium, the age of the culture, etc., and we have found that they vary greatly under different conditions.

He states positively that no reproductive process takes place within the cyst in cultures and, therefore, thinks that others have been mistaken in describing such a process as occurring under any other condition.

There is no summary and the work, on the whole, leaves our ideas still confused in regard to the life cycle of these forms and adds nothing definite to our knowledge of the relationship between the pathogenic and the non-pathogenic organisms.

Craig[2], the second worker who has made a lengthy study of amebae in tropical dysentery since Schaudinn's work, reports in his latest work the study of 1,579 cases of amebic dysentery together with those from many normal individuals, and from those suffering from diseases other than dysentery. He however goes back to the older method of studying the organisms in their natural habitat and considers cultural studies misleading. He thinks Walker's work is of practically no worth in identifying Schaudinn's histolytica because the latter studied only the cultural forms, and he insists upon the importance of studying the organisms fresh from the animal; indeed, he considers this quite sufficient for coming to the conclusions that Schaudinn's work is correct in every particular, that he (Craig) has fully proved it, and that, therefore, the cause of amebic dysentery is a distinct species of ameba corresponding to Schaudinn's *Entameba histolytica.* He says that he is able easily to make the diagnosis from the study of the living material alone. If this is true, of course, is it a very important addition to our knowledge of amebic dysentery, as 65 per cent. to 70 per cent. of the Philippinos have been found to contain amebas in their intestinal tract, without apparently having symptoms (for a long time, at least six months), while those with true dysentery may have intermissions in symptoms of one to two months. In a simple attack of diarrhea in the former case, if only harmless amebas are present, the question of diagnosis is extremely important.

However correct Craig's observations are on the fresh material, we cannot accept his results from fixed and stained material since he used what we consider faulty technic in the preparation of his slides. He allows them to air-dry before fixing, while everyone who has worked with amebas knows that air-dried specimens are unfit for minute study. The slides *must be* fixed while moist. One must consider all of his work on stained specimens as almost worthless because of this technic. He states that he has been unable to get good stained specimens from cultures by his method of staining, whereas if he had prepared his smears properly he would probably have had no trouble. He describes the amebas as dividing by simple amitotic division, and they appear to do so in air-dried smears, but in the fixed moist smears we get the beautiful mitotic figures, which we describe in a future paper.

In regard to making a differential diagnosis from the more obvious characteristics of the organisms, as seen in fresh stools, I may say that while observations made under such conditions may not be sufficiently accurate for determining different species, they may indicate that such species exist, and they may practically be of use in making diagnoses.

It must be remembered, however, that all of these grosser characteristics vary markedly in the same species under different conditions, and for this reason we should be cautious in accepting conclusions based on them until much more work has been done. That the conditions in certain dysenteries are distinctly different may be the cause of a difference in certain characteristics of the amebas, especially the characteristics which are spoken of as of diagnostic significance, namely, size, prominence of nucleus motility, differentiated ectoplasm, presence of red blood cells, color and vacules. In our cultures we have found that all of these characteristics vary markedly in the same culture under different conditions, and though this is not proof that amebas do so in the intestines, it is an indication that they may.

ORIGINAL WORK

In order to be able to judge of the worth of all this work we decided that it would be necessary to study various free-living and parasitic amebas, both in artificial cultures and in animals.

We began our work with two cultures. One, a free-living form isolated by us from potato parings, and the other obtained by Dr. Calkins from a human case of dysentery in the Philippines.

Later, Walker kindly sent us three cultures; one obtained from the Philippines from a case of human amebic dysentery, the other two isolated by him from the cat and guinea pig, respectively.

Two more cultures, isolated by us later, were added to these, one from the feces of a kitten and the other from those of a puppy.

CULTURAL WORK

Culture media—We found that all of the seven cultures grew abundantly at 20° C. on ordinary nutrient agar; also on glucose agar, glycerin agar, and a few other agars we happened to have made up at the time of our experimentation. We use for our routine stock cultures of these amebae the ordinary nutrient agar, transferring every one to two weeks, though if the agar is thinly poured so that the cysts dry quickly they may remain alive an almost indefinite time. One plate of cysts has been living for three years at ordinary room temperature in the dark. At ordinary ice-box temperatures, also, these agar cultures remain alive a long time. The longest time tested is a year, when the cultures were still fully viable.

The bacteria used for their food have been chiefly coli, and dysentery bacilli, but a large number of bacteria isolated from feces have been found to be good food, including *B. typhosus* and *Sp. cholerae.* A few intestinal bacteria and some air bacteria proved to be unsuited at first for their growth, but the amebas quickly became accustomed even to these and grew well later. With some air bacteria they never grew well.

All of the cultures grow very well at blood heat if they are grown with bacteria which do not outgrow them. Under these conditions they grow much more rapidly than at room temperature, and the full-grown organisms are much larger than at a lower temperature. They also may contain many vacules and proceed more rapidly with abundant chromidial formation. In short, the intestinal forms appear much more like the organisms found in their original habitat, and there is little doubt but that they may be made to present a very similar if not an exactly identical appearances.

On the whole, our seven cultures grew much more readily than we were led to expect appearances.

We found, too, that it was a comparatively easy matter to obtain a culture from a single ameba by the following simple procedure.

With a fine platinum loop a separate ameba at the edge of an agar plate culture is drawn across the field under a low power towards the periphery of the plate. When it is well separated from the rest of the culture a disc of agar with the ameba in the center is cut out with a sterile knife, following the circumference of the objective. The disc is then lifted with the flat of the knife and transferred to a fresh agar plate. The bacteria for the growth of the ameba are now added *in small numbers* to the disc, and the plate set aside in the dark at room temperature. Usually the culture develops in three or four days. Such "pure" cultures show no minute morphologic differences in the individual amebas from those first isolated from their natural habitat.

The minute morphologic changes of these "culture" amebas will be made the subject of a special paper, since the work was done in conjunction with Dr. Gary N. Calkins, for whose help and encouragement in the whole work we take this opportunity of expressing our thanks.

Suffice it to say, in regard to the growth and development of all these cultural forms, we have gotten unmistakable appearances of specific mitotic division and of budding in the vegetative stages, and of interesting precystic and intracystic changes which point toward a sexual process, different from any before described. These changes are individual enough to be used as a basis for the making of distinct species in this group. In the formation of chromidium and in budding, these amebas show such a close resemblance to appearances in the Negri and vaccine bodies that a new zest is added to the morphologic study of these latter forms.

It is interesting to note that the two cultures from the Philippines, each isolated from human dysenteric stools, show certain constant morphologic changes which constitute their distinct varieties. These characteristics are: (1) Size. Under similar conditions the one is always distinctly smaller than the other. (2) Shape. The smaller one produces spinous processes on solid media, and stretches itself out in long slender forms. The larger one produces labose pseudopodia on similar culture media and never stretches itself out in slender strings. Both produce labose pseudopodia on fluid and semi-fluid media, but the smaller has more slender forms. The cysts of the larger form are larger and more or less regular in size; those of the smaller are smaller and more irregular in size. Cultures grown from a single ameba show similar characteristics.

Animal Experiments—So far, our animal experiments have been few and rather unsatisfactory as regards minute morphologic study, though we have noted one or two interesting points.

Up to the present only four kittens have been used for experimentation with cultures of amebas. The cultures employed were of three different strains of amebas, one isolated by Miss Wilson from potato parings, one from the intestines of a cat, isolated by Walker (*Am. intestinalis*), and the other, Walker's culture from the Philippines, isolated from a human case of amebic dysentery, each of the two latter grown with *B. coli,* and the former with a yellow air bacillus. The ameba obtained from the potato parings has been shown by Calkins to be *Am. limax*. The kitten inoculated with *Am. intestinalis* showed absolutely no symptoms; while that inoculated with *"Am. coli"* had an attack of typical bloody dysentery. *Ameba limax also produced a short attack of typical bloody dysentery, clinically,* which occurred after each feeding, while the food bacteria alone produced no symptoms. On the cessation of the feedings the kittens gradually recovered.

One, the *Ameba coli* cat, was killed and autopsied after the symptoms had fully disappeared and it showed no lesions.

The *Ameba limax* was recovered from the *Ameba limax* cat, together with a tiny ameba. The *Ameba coli* was not recovered from the *Ameba coli* cat, but from this kitten also a tiny ameba similar to that isolated from the other kitten was obtained.

The *Ameba limax* neither immediately after isolation nor later showed any morphologic differences from the original culture.

We have used five puppies, to each of which was fed agar plate cultures from one of the following strains:

1.	Control	*B. coli.*
2.	Ameba limax	(transplant 42)
3.	Ameba cobaye	(transplant 44)
4.	Ameba coli	(transplant 51)
5.	Ameba coli	(transplant 36)

The feces were examined several times during the two weeks previous to the feeding and in none were ambas found except in No. 3, whose feces contained on two examinations a small number of amebas, which were isolated and found to be a species distinct from the one fed, namely, *Ameba cobaye.*

All of these puppies, except the control, developed a mucous diarrhoea after three to seven days, which was not hemorrhagic and which contained so few of the amebas that none were found in the hanging drops, or smears; and from only two were the organisms recovered in cultures. The puppies did not seem to be ill during the entire three weeks they were fed.

In regard to the cultivation of the two forms, pathogenic and nonpathogenic, there is no proof, as yet, that any form cultivated corresponds with either of Schaudinn's forms, since no one has made a minute comparative study of cultural and fresh organisms from the same case. It is still thought by some (Jürgens, for instance) that the true *histolytica* cannot be made to grow on artificial culture media, while Craig states that he has never been able to grow *Entameba coli* on artificial media; he does not give his technic, however, so we are unable to judge of the worth of his observations.

CONCLUSIONS

In regard to our own work six strains, though the specific ameba of amebic dysentery may not be among them, we at least have been able to demonstrate the following points of interest in regard to them:

1. Amebas of the general type found in amebic dysentery are comparatively easy to cultivate on ordinary nutrient media.
2. Such amebas may be easily grown from a single ameba.
3. In the cultural forms definite mitotic division occurs in the the vegetative stage as the only mode of binary division.
4. Budding may occur throughout the vegetative stage, in the cultural forms.
5. In cultures, too, chromidial changes occur in the precystic and cystic forms which point towards a specific sexual process. Some of the appearances in these stages are similar to certain forms of the Negri bodies.
6. A culture of a saprophytic ameba (*Ameba limax*) produced the clinical symptoms of bloody dysentery in a kitten. The organism was recovered from the stools, with no morphologic changes in the first cultures isolated.

In summarizing, we may say that the work so far seems to indicate that cultural forms of intestinal amebas do not differ materially from the forms in their natural habitat, and that there are probably several varieties and possibly more than one species, which produce amebic dysentery, as there are undoubtedly several varieties and species of non-pathogenic amebas in the human intestines.

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